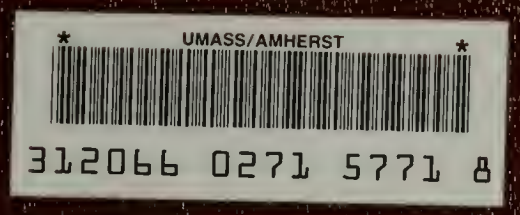

STANDARD OPERATING PROCEDURES

BIOMONITORING PROGRAM 1990

Massachusetts Department of Environmental Protection
DIVISION OF WATER POLLUTION CONTROL
Arleen O'Donnell, Acting Director

Publication # 16,326 - 147 pgs - 25 cps. 5/90 CR
Approved by: Ric Murphy, State Purchasing Agent

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BIOMONITORING PROGRAM

STANDARD OPERATING PROCEDURES

1990

Technical Services Branch
Massachusetts Division of Water Pollution Control
Department of Environmental Protection
Westborough

Executive Office of Environmental Affairs
John P. DeVillars, Secretary

Department of Environmental Protection
Daniel S. Greenbaum, Commissioner

Division of Water Pollution Control
Arleen O'Donnell, Acting Director

May 1990

TITLE: Biomonitoring Program Standard Operating Procedures

DATE: May 1, 1990

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BIOMONITORING PROGRAM

1.0 INTRODUCTION AND PURPOSE

1.0 INTRODUCTION AND PURPOSE

It is the goal of the Federal Clean Water Act (PL 95-217) to restore and maintain the biological integrity of the nation's waters. Biological monitoring provides the most reliable measure of the attainment of this goal, i.e., water quality that provides for the protection and propagation of fish, shellfish and wildlife.

Sampling and analyzing aquatic life provides information on water quality that can easily escape standard physico-chemical sampling. The organisms themselves are efficient in-stream monitors, for their lives reflect the cumulative impact of pollution on the waterbody. They are valuable in revealing transient pollution episodes such as oil spills and brief dissolved oxygen sags. For the same reason they are the best means of measuring long term trends in a waterbody. In addition, the presence of specific indicator organisms may infer the presence of particular chemicals not included in routine analysis or in quantities below detection limits of chemical testing.

Aquatic biota are usually collected and analyzed by community. These communities include plankton, periphyton, macrophyton, macroinvertebrates and fish. The communities are used alone or in combinations to assess specific water quality problems such as thermal pollution, toxics, and eutrophication. The analysis of the samples includes taxonomic identification for diversity indices, water quality indices, trophic level and indicator organism analysis. Plant pigments are extracted for chlorophyll analysis and animal tissues are tested for bioconcentration of chemicals. The overall health and appearance of the organisms is used to detect chronic toxicity and genotoxic effects (carcinogens, mutagens and teratogens). Standard laboratory organisms are also used *in situ* and *in vitro* to measure toxicity. Bacteria, algae, macroinvertebrates and fish are all commonly used for this purpose.

Biological monitoring can be more cost effective than chemical screening, more reliable at measuring total pollutant loads, more sensitive to extreme conditions and more faithful to the goal of the Act, than other forms of monitoring. However, the relationship between the biota and the environment is subtle and complex and by no means completely understood. Results of biological investigations are often qualitative, and even quantitative studies are open to interpretation. Therefore biological monitoring data are used to complement physico-chemical data and not replace them.

The methods of monitoring and analysis are evolving and may differ among investigators. At best, procedures used by the Division of Water Pollution Control are fully documented in this Standard Operating Procedures document, so that those attempting interpretation will be fully informed, and temper their conclusions accordingly.

2.0 BIOMONITORING SURVEY PROGRAM ELEMENTS

2.1 STREAM CLASSIFICATION

2.1.1 INTRODUCTION AND PURPOSE

This program has been developed to systematically sample and classify the Commonwealth's rivers and streams. Each survey qualitatively provides documentation of a specific watercourse's physical and chemical characteristics and predominant biological components. These data can be used - on a stream or site-specific basis - to determine water-use classifications in accordance with Massachusetts Surface Water Quality Standards.

2.1.2 OBJECTIVES

1. To identify, demonstrate, and standardize methods and procedures for the collection and analyses of stream habitat data;

2. to characterize rivers, streams, and related aquatic habitats (e.g., river impoundments) hydrophysically and chemically;

3. to qualitatively document the dominant floral and faunal components - or communities - of streams and stream-side habitats;

4. to segment and classify rivers and streams into major habitats for the purpose of water-use designation;

5. to provide supplementary information to other programs to aid in regulatory and enforcement actions, and evaluating special problems; and

6. to collect and reference plant and animal specimens for future study, and determine their state-wide distribution.

2.1.3 APPROACH

Preliminary planning and analysis first divides the river or stream into longitudinal zones - or subsystems, i.e., tidal, lower perennial, upper perennial, intermittent, and others (e.g., canals, ditches) - according to morphometric and hydrologic characteristics derived from USGS topographic maps. Physico-chemical and biological field collections are made, in most instances, at locations - or sites - determined after initial evaluation and field reconnaissance (see: "Data Record Sheets"). Specific sampling locations are arranged to cover significant and representative lotic-water and other related macrohabitats. Field dates, particularly for biological sampling, are generally during the period April to October, in order to take advantage of plant and animal availability. All field sampling is qualitative in nature, unless special needs dictate otherwise. Data collected are recorded for each community on individual standard field sheets (see: "Biological Field and Laboratory Methods").

2.1.4 PARAMETRIC COVERAGE

Physical and chemical data are collected, including: stream reach width and depth; stream reach and floodplain substrate character; stream temperature; water transparency; and water chemistry. Sampling of phytoplankton and periphyton, aquatic vascular plants, streamside and riparian vegetation, and aquatic macroinvertebrates is performed at each site.

2.1.5 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION FIELD RECONNAISSANCE DATA RECORD

RIVER:

DRAINAGE:

MUNICIPALITY:

SITE:

COLLECTOR(S):

DATE:

TOPOGRAPHIC MAP(S):

WEATHER/ATMOSPHERIC CONDITIONS:

Air Temperature -

Wind Force/Direction -

Cloud Cover -

Precipitation -

PHYSICO-CHEMICAL

Flow Regime -

Substrate Character -

Light -

Other -

PHYTOPLANKTON

Channel -

Riffle -

Pool -

Other -

PERIPHYTON

Channel -

Riffle -

Pool -

Other -

AQUATIC VEGETATION

Channel -

Riffle -

Pool -

Other -

RIPARIAN VEGETATION

Trees -

Shrubs -

Herbaceous -

Grasses -

Other -

AQUATIC INVERTEBRATES

Channel -

Riffle -

Pool -

Other -

GENERALIZED SITE MAP

OTHER REMARKS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION FIELD DATA RECORD

RIVER:	SITE:
DRAINAGE:	COLLECTOR(S):
MUNICIPALITY:	DATE:
TOPOGRAPHIC MAP(S):	TIME SAMPLING DURATION:

WEATHER/ATMOSPHERIC CONDITIONS:

AIR TEMPERATURE

WIND

Force -
Direction -

CLOUD CLASSIFICATION

Low
Middle
High
Percent Sky Coverage

PRECIPITATION (including previous day's)

DATA COLLECTIONS:

PHYSICAL	HYDROLOGIC
WATER	BIOLOGICAL

OTHER OBSERVATIONS:	INSTRUMENT CALIBRATION

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION PHYSICO-CHEMICAL FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION(S):	SAMPLE NUMBER(S):

FLOW REGIME	SUBSTRATE (% AREAL COVER)		CHEMICAL		
Intermittent	Inorganic	Organic			
Upper Perennial			D.O.		
Lower Perennial	Bed Rock	Detritus	pH		
Tidal	Boulders	Fibrous Peat	Alk		
	Rubble	Pulpy Peat	Spec. cond.		
WATER TEMPERATURE	Gravel	Muck	NH_3	NO_3	ON
	Sand		TP	OP	
SECCHI DISK DEPTH	Clay		BOD	COD	TOC
	Marl		TS	TSS	TDS
SOLAR RADIATION			Metals		
			Other		
CHANNEL MORPHOLOGY					
Slope					
Vegetation					
Cover					

WIDTH	SAMPLE EQUIPMENT/METHODS:
VELOCITY 0.2 0.6 0.8	SITE MAP:
DEPTH	

D < 2.5 ft, V 0.6	D > 2.5 ft, V 0.2 + V 0.8	D x V $\geq$9 Not Wadeable

2.1.6 REFERENCES

1. Cowardin, L.M., V. Carter, F.C. Golet, and E.T. LaRoe. 1979. Classification of Wetlands and Deepwater Habitats of the United States. FWS/OBS 79/31. Office of Biological Services, United States Fish and Wildlife Service, Washington, D.C. vi + 103 p.

2. Marmelstein, A. 1978. Classification, Inventory, and Analysis of Fish and Wildlife Habitat: Proceedings of a National Symposium held at Phoenix, Arizona, 24-27 January 1977. FWS/OBS-78/76. Office of Biological Services, U.S. Fish and Wildlife Service, Washington, D.C. vi + 604 p.

3. Platts, W.S., W.F. Megahan, and G.W. Minshall. 1983. Methods for Evaluating Stream, Riparian, and Biotic Conditions. General Technical Report INT-138. Intermountain Forest and Range Experiment Station, United States Forest Service, Ogden, Utah. ii + 70 p.

4. Sather, J.H., (ed). 1976. Proceedings of the National Wetland Classification and Inventory Workshop held at the University of Maryland, College Park, Maryland, 20-23 July 1975. FWS/OBS-76/09. Office of Biological Services, U.S. Fish and Wildlife Service, Washington, D.C. vi + 110 p.

5. Terrell, T.T. and W.J. McConnell, (eds). 1978. Stream Classification - 1977: Proceedings of a Workshop held at Pingree Park, Colorado, 10-13 October 1977. FWS/OBS-78/23. Biological Services Program, U.S. Fish and Wildlife Service, Fort Collins, Colorado. iv + 45 p.

2.2 AQUATIC MACROINVERTEBRATE RAPID BIOASSESSMENT

2.2.1 INTRODUCTION AND PURPOSE

Macroinvertebrate rapid bioassessment (MRB) surveys involve the use of qualitative and semiquantitative sampling methods designed to minimize laboratory time requirements for taxonomic identification and enumeration of aquatic macroinvertebrate organisms.

2.2.2 OBJECTIVES

1. To provide standardized methods and procedures for assessing the impacts of toxic and conventional organic pollution on aquatic macroinvertebrates;

2. to obtain reliable biological water quality information to supplement the collection of standard physico-chemical water quality data; and

3. to provide the basis for making relative comparisons pertaining to water quality conditions between sampling stations and/or to document long-term trends at fixed sites.

2.2.3 APPROACH

While rapid bioassessments make use of the qualitative analysis of periphyton, aquatic and wetland vegetation, and fish communities, specific semi-quantitative sampling and analytical methods have been developed for use in assessing the macroinvertebrate community.

An upstream-downstream sampling regime is employed whereby known or suspected sources of pollution are bracketed by sampling stations. Selected aquatic communities are assessed and compared with unimpacted control (or reference) communities. Conclusions relative to water quality condition are drawn from a knowledge of the environmental requirements and pollution ecology of the individual taxa or assemblages encountered.

For macroinvertebrate rapid bioassessment the components of a 100 organism subset are identified to genus or species level whenever possible. The taxonomic data are then compiled to determine the status of the various criteria used to rank water quality. These criteria include:

1. Species richness;

2. distribution "balance";

3. the EPT value;

4. percent contribution, pollution tolerances, and feeding habits of the five numerically dominant species;

5. Hilsenhoff Biotic Index (HBI).

Field observations are also considered, as they often reveal important factors contributing to the quality of the benthic community.

Species richness, the number of different kinds of organisms present, will tend to decrease in response to pollution while the distribution of individuals becomes uneven, or unbalanced. That is to say, under the influence of pollution benthic macroinvertebrate communities become less diverse, with the majority of individuals falling into fewer taxa (Tarzwell and Gaufin 1953, Bartsch and Ingram 1959, Weber 1973, Hawkes 1979, and Welch 1980). By examining the relative contribution of the five numerically dominant taxa the evenness of the distribution can be judged.

The pollution tolerances of the dominant community members can be revealing as to the degree of pollution impacting a stream. Likewise, the number of species present from the orders Ephemeroptera, Plecoptera, and Trichoptera can be tabulated to formulate the "EPT value." These orders are composed of species that are regarded as intolerant or facultative in response to enrichment with conventional pollutants--Plecoptera are all intolerant, Ephemeroptera and Trichoptera have both intolerant and facultative members (Weber 1973, Hilsenhoff 1982). Also of importance are the feeding habits of the dominant taxa, as these will reflect community shifts to exploit the food source available, e.g., a filter feeding community downstream of an effluent high in suspended solids.

Hilsenhoff (1982) developed an index (HBI) based on the tolerances of aquatic macroinvertebrates to pollution with conventional organics. While his sampling protocol was similar to the one used here, he restricted his analysis to aquatic arthropods dependent on dissolved oxygen. The MRB, on the other hand, makes use of aquatic annelids and mollusks for the information they may contribute in attempts to evaluate the impacts of various types of pollution. Consequently, if the HBI is to be used as part of the MRB it becomes necessary to assign tolerance values to organisms excluded by Hilsenhoff as well as any regionally unique aquatic arthropod taxa that otherwise would have been included by Hilsenhoff. Since Hilsenhoff's tolerance values range from zero (intolerant) to five (tolerant) and most literature provides information on pollution tolerances as tolerant, facultative, and intolerant, assigning new values is difficult. Lacking any better information the values are assigned as: intolerant=1, facultative=2.5, and tolerant=4. These modifications surely weaken the reliability of the HBI, if not by using dubious tolerance values, then at least by virtue of eliminating the sensitivity to the extremes. Nonetheless, with these considerations in mind the HBI is retained in the MRB because if the index value falls at one of the extremes it indicates either very little DO stress (HBI<2) or very serious DO stress (HBI>4).

The MRB guidelines identify the range of characteristics indicative of different levels of pollution as follows:

1. Non-Impacted - Diverse fauna, at least 30 species in riffle habitats. Biotic index less than 2.00. Mayflies, stoneflies, and caddisflies are well-represented, EPT value greater than 10. Dominant species are intolerant or facultative; no species comprises more

than 25% of the individuals; oligochaete worms comprise less than 20% of the individuals.

2. Slightly Impacted - Species richness usually 20-30. Biotic index 2.00-3.00. Mayflies and stoneflies may be restricted, EPT value 6-10. Dominant species are mostly facultative. Fauna often not so well balanced, often with one species comprising more than 25% of the individuals; oligochaete worms may comprise more than 20% of the individuals.

3. Moderately Impacted - Species richness 10-20. Biotic index 3.00-4.00. Mayflies and stoneflies rare or absent, caddisflies often restricted, EPT value 2-5. Dominant species are facultative or tolerant. Oligochaetes often comprise at least 20% of the individuals.

4. Severely Impacted - Species richness less than 10. Biotic index greater than 4.00. Mayflies, stoneflies, and caddisflies rare or absent, EPT value 0-1. Fauna often restricted to midges and worms. Dominant species are almost all tolerant. Fauna usually greatly imbalanced, with dominant species comprising more than 35% of the individuals.

These are generalizations about complex ecosystems and may not always result in complete agreement of all parameters. In such cases it is necessary to select a category based on a consensus of the majority of indicators. It is also necessary to consider the integrity of each component so that those possibly influenced by factors other than pollution can be de-emphasized, or if appropriate, eliminated from the assessment. For instance, a data set may contain 21 species, no species representing more than 25% of the community, oligochaetes comprising 21%, an EPT value of three, an HBI of 3.25, with four of the five dominant species being facultative, and the fifth being tolerant. Knowing that the data set includes significant numbers of aquatic annelids and mollusks, the HBI should not weigh heavily in the analysis. A review of the other criteria would tend toward a rating of "slightly impacted" for this hypothetical community.

2.2.4 PARAMETRIC COVERAGE

Rapid assessment surveys include, at a minimum, semi-quantitative aquatic macroinvertebrate sampling and water temperature determinations. However, qualitative analyses of the algae, macrophyte, and fish communities may also be conducted. Often, flow measurements, substrate characterization, and water chemistry sampling are conducted to supplement the results of biological sampling.

2.2.5 AQUATIC MACROINVERTEBRATES AND ASSIGNED TOLERANCE VALUES

TAXON NAME	TOLERANCE VALUE
ANNELIDA	
OLIGOCHAETA (AQUATIC EARTHWORMS)	
LUMBRICULIDA	
LUMBRICULIDAE	4.0N
LUMBRICULUS SP.	4.0N
HAPLOTAXIDA	
ENCHYTRAEIDAE	4.0N
NAIDIDAE	
DERO SP.	5.0B
HAEMONAIS WALDVOGELI	2.5N
NAIS SP.	
NAIS BEHNINGI	2.5N
NAIS COMMUNIS	4.0B
NAIS PARDALIS	4.0B
NAIS VARIABILIS	5.0B
OPHIDONAIS SERPENTINA	3.0N
SPECARIA SP.	4.0N
TUBIFICIDAE	
AULODRILUS SP.	4.0B
IMMATURE W/O CAPILLIFORM SETAE	5.0B
IMMATURE W/O CAPILLIFORM SETAE TYPE 1	5.0B
IMMATURE W/O CAPILLIFORM SETAE TYPE 2	5.0B
IMMATURE WITH CAPILLIFORM SETAE	5.0B
LIMNODRILUS SP.	5.0B
LIMNODRILUS HOFFMEISTERI	5.0N
TUBIFEX SP.	5.0N
TUBIFEX TUBIFEX	5.0N
HIRUDINEA (LEECHES)	
RHYNCHOBDELLIDA	
GLOSSOPHONIIDAE	
HELOBDELLA STAGNALIS	4.0N
PHARYNGOBDELLIDA	
ERPOBDELLIDAE	4.0N
ERPOBDELLA PUNCTATA	4.0N
ARTHROPODA	
CRUSTACEA	
ISOPODA (SOW BUGS)	
ASELLIDAE	
ASELLUS SP.	
ASELLUS COMMUNIS	4.0N
AMPHIPODA (SCUDS)	
HYALELLIDAE	
HYALELLA SP.	
HYALELLA AZTECA	3.0N
GAMMARIDAE	
GAMMARUS SP.	
GAMMARUS FASCIATUS	2.5N
ARACHNIDA	
ACARI HYDRACARINA (WATER MITES)	1.0N

TAXON NAME	TOLERANCE VALUE
INSECTA	
EPHEMEROPTERA (MAYFLIES)	2.5N
SIPHLONURIDAE	
AMELETUS SP.	0.0H
SIPHLONURUS SP.	2.0H
BAETIDAE	2.5N
BAETIS SP.	2.5N
BAETIS SP. 1 (2 CAUDAL FILAMENTS)	2.5N
BAETIS SP. 2 (SHORT TERMINAL FILAMENT)	2.5N
BAETIS SP. 3 (3 CAULDAL FILAMENTS)	2.5N
BAETIS FRONDALIS	2.0H
BAETIS INTERCALARIS	3.0H
BAETIS LEVITANS	2.5N
BAETIS PHOEBUS	2.5N
BAETIS VAGANS	1.0H
CALLIBAETIS SP.	3.0H
CENTROPTILUM SP.	1.0H
CLOEON SP.	2.0H
PSEUDOCLOEON SP.	2.0N
PSEUDOCLOEON NR. CAROLINA	1.0H
OLIGONEURIDAE	
ISONYCHIA SP.	2.0H
HEPTAGENIIDAE	
EPEORUS (IRON) SP.	0.0J
HEPTAGENIA SP.	
HEPTAGENIA HEBE/APHRODITE	1.0H
HEPTAGENIA MACULIPENNIS	2.0J
HEPTAGENIA PULLA	0.0H
RHITHROGENA SP.	0.0H
STENONEMA SP.	2.0N
STENONEMA MEDIOPUNCTATUM	2.0H
STENONEMA MODESTUM	0.0H
STENONEMA PUDICUM	0.5N
STENONEMA PULCHELLUM	1.0H
STENACRON SP.	
STENACRON INTERPUNCTATUM S.S.	3.0H
STENACRON INTERPUNCTATUM FRONTALE	3.0N
EPHEMERELLIDAE	
ATTENELLA SP.	
ATTENELLA ATTENUATA	1.0H
DANNELLA SP.	1.0H
DANNELLA SIMPLEX	1.0H
DRUNELLA SP.	0.0H
EPHEMERELLA SP.	1.0H
EPHEMERELLA DOROTHEA	0.0H
EPHEMERELLA INVARIA	1.0H
EPHEMERELLA NEEDHAMI	1.0H
EURYLOPHELLA SP.	2.0N
SERRATELLA DEFICIENS	1.0H
TRICORYTHIDAE	
TRICORYTHODES SP.	2.0H
CAENIDAE	
BRACHYCERCUS SP.	2.0H

TAXON NAME	TOLERANCE VALUE
CAENIS SP.	3.0H
LEPTOPHLEBIIDAE	
CHLOROTERPES SP.	2.0J
HABROPHLEBIODES SP.	1.0J
HABROPHLEBIODES AMERICANA	2.0H
LEPTOPHLEBIA SP.	2.0H
PARALEPTOPHLEBIA SP.	1.0H
POTAMANTHIDAE	
POTAMANTHUS SP.	2.0H
EPHEMERIDAE	
EPHEMERA SP.	
EPHEMERA SIMULANS	1.0H
EPHEMERA VARIA	1.0J
HEXAGENIA SP.	3.0H
HEXAGENIA LIMBATA	3.0J
ODONATA (DRAGONFLIES AND DAMSELFLIES)	
ANISOPTERA (DRAGONFLIES)	
CORDULEGASTRIDAE	
CORDULEGASTER SP.	
CORDULEGASTER MACULATA	1.0H
CORDULIIDAE	
GOMPHIDAE	
DROMOGOMPHUS SP.	3.0J
GOMPHUS SP.	2.0H
OPHIOGOMPHUS SP.	1.0H
AESCHNIDAE	
AESCHNA SP.	3.0H
BASIAESCHNA SP.	
BASIAESCHNA JANATA	2.0H
BOYERIA SP.	
BOYERIA VINOSA	1.0H
MACROMIIDAE	
MACROMIA SP.	2.0J
LIBELLULIDAE	
PERITHEMIS SP.	2.5N
PLATHEMIS SP.	
PLATHEMIS LYDIA	2.5N
ZYGOPTERA (DAMSELFLIES)	
CALOPTERYGIDAE	
AGRION SP.	
CALOPTERYX SP.	2.0H
CALOPTERYX MACULATUM	2.0H
LESTIDAE	
LESTES SP.	3.0H
LESTES RECTANGULARIS	3.0H
LESTES VIGILAX	3.0H
COENAGRIONIDAE	
ARGIA SP.	2.5N
ARGIA MOESTA	2.0H
ENALLAGMA SP.	3.0H
ENALLAGMA CIVILE	3.0H
ENALLAGMA EXSULANS	3.0H
ENALLAGMA GEMINATUM	3.0H

TAXON NAME	TOLERANCE VALUE
ENALLAGMA HAGENI-EBRIUM GROUP	3.0H
ENALLAGMA SIGNATUM	3.0H
ISCHNURA SP.	
ISCHNURA VERTICALIS	4.0H
PLECOPTERA (STONEFLIES)	
PTERONARCIDAE	
PTERONARCYS SP.	1.0H
NEMOURIDAE	
NEMOURA SP.	
NEMOURA VENOSA	0.0H
LEUCTRIDAE	
LEUCTRA SP.	0.0H
PERLIDAE	
ACRONEURIA SP.	0.0H
ACRONEURIA ABNORMIS	0.0H
ACRONEURIA NR. CAROLINENSIS	0.0H
ACRONEURIA GEORGIANA	0.0H
ACRONEURIA LYCORIAS	0.0H
NEOPERLA SP.	2.0J
NEOPERLA CLYMENE	1.0H
PARAGNETINA SP.	1.0N
PERLESTA SP.	
PERLESTA PLACIDA	2.0H
PHASGANOPHORA SP.	
PHASGANOPHORA CAPITATA	0.0H
HEMIPTERA (TRUE BUGS)	
CORIXIDAE (WATER BOATMEN)	4.0N
MEGALOPTERA (DOBSONFLIES & ALDERFLIES)	
SIALIDAE (ALDERFLIES)	
SIALIS SP.	2.0H
CORYDALIDAE (DOBSONFLIES)	2.0H
CHAULOIDES SP.	2.0H
CORYDALUS SP.	
CORYDALUS CORNUTUS	2.0H
NIGRONIA SP.	1.0N
NEUROPTERA	
SISYRIDAE (SPONGILLA FLIES)	
SISYRA SP.	1.0N
TRICHOPTERA (CADDISFLIES)	2.5N
PHILOPOTAMIDAE	1.5N
CHIMARRA SP.	1.5N
CHIMARRA ATERRIMA	2.0H
CHIMARRA OBSCURA	2.0H
PSYCHOMYIIDAE	
PSYCHOMYIA SP.	2.0N
POLYCENTROPIDAE	2.5N
CERNOTINA SP.	
NEURECLIPSIS SP.	4.0H
NYCTIOPHYLAX SP.	1.0H
PHYLOCENTROPUS SP.	2.0H
PHYLOCENTROPUS PLACIDUS	1.0H
HYDROPSYCHIDAE	2.5N
CHEUMATOPSYCHE SP.	3.0H

TAXON NAME	TOLERANCE VALUE
DIPLECTRONA SP.	
DIPLECTRONA MODESTA	0.0H
HYDROPSYCHE SP.	2.5N
HYDROPSYCHE BETTENI	3.0H
HYDROPSYCHE CUANIS	3.0H
HYDROPSYCHE SIMULANS	3.0H
(SYMPHITOPSYCHE GROUP)	2.5N
HYDROPSYCHE BIFIDA	3.0H
HYDROPSYCHE PHALERATA	1.0H
HYDROPSYCHE RIOLA	2.0H
HYDROPSYCHE SLOSSONAE	2.0H
HYDROPSYCHE SPARNA	1.0H
MACROSTEMUM SP.	
MACROSTEMUM ZEBRATUM	2.0H
RHYACOPHILIDAE	
RHYACOPHILA SP.	0.0H
RHYACOPHILA FUSCULA	0.0H
GLOSSOSOMATIDAE	
AGAPETUS SP.	1.0H
GLOSSOSOMA SP.	1.0H
PROTOPTILA SP.	1.0H
HYDROPTILIDAE	3.0N
AGRAYLEA SP.	3.0H
HYDROPTILA SP.	3.0H
LEUCOTRICHIA SP.	3.0H
LEUCOTRICHIA PICTIPES	3.0H
OCHROTRICHIA SP.	3.0H
OXYETHIRA SP.	2.0N
PHRYGANEIDAE	
AGRYPNIA SP.	2.0H
PHRYGANEA SP.	2.0N
PTILOSTOMIS SP.	2.0N
BRACHYCENTRIDAE	
BRACHYCENTRUS SP.	0.5N
MICRASEMA SP.	0.5N
LEPIDOSTOMATIDAE	
LEPIDOSTOMA SP.	1.0H
LIMNEPHILIDAE	
LIMNEPHILUS SP.	2.0H
NEOPHYLAX SP.	2.0H
PYCNOPSYCHE SP.	2.0H
ODONTOCERIDAE	
PSILOTRETA SP.	0.0N
MOLANNIDAE	
MOLANNA SP.	1.0H
MOLANNA UNIOPHILA	1.0H
HELICOPSYCHIDAE	
HELICOPSYCHE SP.	2.0H
HELICOPSYCHE BOREALIS	2.0H
LEPTOCERIDAE	
CERACLEA SP.	2.0H
CERACLEA TARSI-PUNCTATUS	2.0H
LEPTOCERUS SP.	

TAXON NAME	TOLERANCE VALUE
LEPTOCERUS AMERICANUS	2.0H
MYSTACIDES SP.	2.0H
NECTOPSYCHE SP.	2.0H
OECETIS SP.	2.0H
OECETIS AVARA	2.0H
OECETIS CINERASCENS	2.0H
OECETIS INCONSPICUA	2.0H
SETODES SP.	2.0H
TRIAENODES SP.	2.0H
TRIAENODES ABA	2.0H
TRIAENODES NR. MARGINATA	2.0H
LEPIDOPTERA (BUTTERFLIES AND MOTHS)	
PYRALIDAE	
NYMPHULA SP.	1.0H
PARAGYRACTIS SP.	2.0H
PARAPOYNX SP.	1.0H
COLEOPTERA (BEETLES)	
GYRINIDAE (WHIRLIGIGS)	
DINEUTUS SP. (LARVAE ONLY)	2.0H
DINEUTUS CILIATUS (LARVAE ONLY)	2.0H
GYRINUS SP. (LARVAE ONLY)	2.0H
GYRINUS FRATERNUS (LARVAE ONLY)	2.0H
GYRINUS VENTRALIS (LARVAE ONLY)	2.0H
HALIPILDAE	
PELTODYTES SP.	4.0N
DYTISCIDAE (PREDACIOUS DIVING BEETLES)	
AGABUS SP.	4.0N
DERONECTES SP.	4.0N
HYDROPHILIDAE (WATER SCAVENGER BEETLES)	
BEROSUS SP.	4.0N
PARACYMUS SP.	4.0N
PSEPHENIDAE (WATER PENNIES)	
ECTOPRIA SP.	2.0H
ECTOPRIA NERVOSA	2.0H
PSEPHENUS SP.	2.0N
PSEPHENUS HERRICKI	2.0H
DRYOPIDAE	
HELICHUS SP.	2.0H
ELMIDAE (RIFFLE BEETLES)	
ANCYRONYX SP.	
ANCYRONYX VARIEGATA	2.0H
DUBIRAPHIA SP.	
DUBIRAPHIA BIVITTATA	2.0H
MACRONYCHUS SP.	
MACRONYCHUS GLABRATUS	2.0H
MICROCYLLOEPUS SP.	
MICROCYLLOEPUS PUSILLUS	1.0H
OPTIOSERVUS SP. (LARVAE ONLY)	2.0H
OPTIOSERVUS FASTIDITUS (LARVAE ONLY)	2.0H
OULIMNIUS SP.	2.5N
OULIMNIUS LATIUSCULUS	2.5N
PROMORESIA SP.	
PROMORESIA TARDELLA	1.0N

TAXON NAME	TOLERANCE VALUE
STENELMIS SP. (ALL LARVAE)	3.0H
STENELMIS SP. 1	2.5N
STENELMIS SP. 2	2.5N
STENELMIS CRENATA GR. (LARVAE ONLY)	3.0H
PTILODACTYLIDAE	
CURCULIONIDAE	5.0N
DIPTERA (TRUE FLIES)	
BLEPHARICERIDAE	
BLEPHARICERA SP.	
BLEPHARICERA TENUIPES	0.0H
TIPULIDAE (CRANE FLIES)	
ANTOCHA SP.	2.0H
DICRANOTA SP.	2.0H
HELIUS SP.	3.0H
HEXATOMA SP.	3.0H
HEXATOMA (ERIOCERA) SPINOSA	3.0H
LIMONIA SP.	2.0H
PEDICIA SP.	2.0H
PILARIA SP.	3.0N
TIPULA SP.	2.0H
CULICIDAE (MOSQUITOES)	
ANOPHELES SP.	5.0N
CULEX SP.	5.0N
CULEX PIPIENS	5.0N
CHAOBORIDAE (PHANTOM MIDGES)	
CHAOBORUS SP.	4.0H
PSYCHODIDAE (MOTH FLIES)	
PSYCHODA SP.	5.0H
CERATOPOGONIDAE (BITING MIDGES)	3.0N
BEZZIA SP.	3.0H
CULICOIDES SP.	4.0H
FORCIPOMYIA SP.	1.0H
PALPOMYIA SP	
PALPOMYIA/PROBEZZIA SP.	3.0H
PROBEZZIA SP.	3.0H
SIMULIIDAE (BLACK FLIES)	
SIMULIUM SP.	3.0N
SIMULIUM VENUSTUM	3.0H
SIMULIUM VITTATUM	4.0H
CHIRONOMIDAE (MIDGES)	3.0N
TANYPODINAE	2.5N
ABLABESMYIA SP.	3.0H
ABLABESMYIA AURIENSIS	3.0H
ABLABESMYIA JUNTA	3.0H
ABLABESMYIA MALLOCHI	3.0H
ABLABESMYIA PELEENSIS	3.0H
ARCTOPELOPIA SP.	3.0H
ARCTOPELOPIA SP. 1	3.0H
ARCTOPELOPIA ALBA	3.0H
CLINOTANYPUS SP.	3.0H
CLINOTANYPUS SP. 3	3.0H
COELOTANYPUS SP.	2.0H
CONCHAPELOPIA SP.	3.0H

TAXON NAME	TOLERANCE VALUE
CONCHAPELOPIA/ARCTOPELOPIA	3.0H
DEROTANYPUS SP.	2.5N
DJALMABATISTA SP.	3.0N
GUTTIPELOPIA SP.	3.0H
MEROPELOPIA SP.	3.0H
NATARSIA SP.	3.0H
NATARSIA TYPE 1	3.0H
NATARSIA TYPE 2	3.0H
NILOTANYPUS SP.	3.0H
PENTANEURA SP.	2.0H
PENTANEURA SP.1	2.0H
PENTANEURA SP.2	2.0H
PROCLADIUS SP.	3.0H
PSECTROTANYPUS SP.	3.0H
RHEOPELOPIA SP.	3.0H
TANYPUS SP.	4.0H
TELOPELOPIA SP.	3.0H
THIENEMANNIMYIA GROUP	3.0H
DIAMESINAE	
DIAMESA SP.	2.0H
PAGASTIA SP.	2.0H
POTTHASTIA SP.	2.0H
POTTHASTIA GAEDII GR.	2.0H
POTTHASTIA LONGIMANUS	2.0H
SYMPOTTHASTIA SP.	2.0H
ORTHOCLADIINAE	3.5N
BRILLIA SP.	3.0H
BRILLIA FLAVIFRONS	3.0H
BRILLIA PAR	3.0H
BRILLIA PAR (VAR.)	3.0H
BRILLIA SERA	3.0H
CARDIOCLADIUS SP.	3.0H
CARDIOCLADIUS ALBIPLUMUS	2.0H
CARDIOCLADIUS OBSCURUS	3.0H
CORYNONEURA SP.	2.0H
CORYNONEURA NR. CELERIPES	2.0H
CORYNONEURA TARIS	2.0H
CRICOTOPUS SP.	4.0H
CRICOTOPUS/ORTHOCLADIUS SP.	4.0N
CRICOTOPUS SP. 2	4.0H
CRICOTOPUS SP. 3	4.0H
CRICOTOPUS BICINCTUS	4.0H
CRICOTOPUS BICINCTUS GROUP	4.0H
CRICOTOPUS CYLINDRACEUS/FESTIVELLUS GROUP	4.0H
CRICOTOPUS EXILUS	4.0H
CRICOTOPUS FLAVOCINCTUS (NR.)	4.0H
CRICOTOPUS FUGAX	4.0H
CRICOTOPUS INTERSECTUS GROUP	4.0H
CRICOTOPUS JUNUS	4.0H
CRICOTOPUS SLOSSONAE	4.0H
CRICOTOPUS SYLVESTRIS	4.0H
CRICOTOPUS SYLVESTRIS GROUP	4.0H
CRICOTOPUS SYLVESTRIS/INTERSECTUS	4.0H

TAXON NAME	TOLERANCE VALUE
CRICOTOPUS TREMULUS	4.0H
CRICOTOPUS TREMULUS GROUP	4.0H
CRICOTOPUS TRIANNULATUS	4.0H
CRICOTOPUS TRIFASCIA	4.0H
CRICOTOPUS NR. TRIFACIATUS	4.0H
CRICOTOPUS VIERRIENSIS	4.0H
EUKIEFFERIELLA SP.	2.0H
EUKIEFFERIELLA SP. 1/SIMILIS	2.0H
EUKIEFFERIELLA SP. 2	2.0H
EUKIEFFERIELLA BREHMI GROUP	2.0H
EUKIEFFERIELLA BREVINERVIS	2.0H
EUKIEFFERIELLA BREVICALCAR GROUP	2.0H
EUKIEFFERIELLA CLARIPENNIS GROUP	2.0H
EUKIEFFERIELLA CYANEA GROUP	2.0H
EUKIEFFERIELLA DEVONICA GROUP	2.0H
EUKIEFFERIELLA GRACEI GROUP	2.0H
EUKIEFFERIELLA PSEUDOMONTANA GROUP	2.0H
EUKIEFFERIELLA RECTANGULARIS GROUP	2.0H
E. SIMILIS GR. (NOW CARDIOCLADIUS ALB.)	2.0H
EUKIEFFERIELLA SORDENS	2.0H
HETEROTRISSOCLADIUS SP.	2.0H
NANOCLADIUS SP.	1.0H
NANOCLADIUS NR. MINIMUS	1.0H
NANOCLADIUS NR. MINIMUS	1.0H
ORTHOCLADIUS SP.	3.0H
ORTHOCLADIUS ANNECTANS	3.0H
ORTHOCLADIUS CARLATUS	3.0H
ORTHOCLADIUS OBUMBRATUS	3.0H
ORTHOCLADIUS ROBACKI	3.0H
ORTHOCLADIUS TYPE 3	3.0H
PARACHAETOCLADIUS SP.	1.0B
PARACHAETOCLADIUS SP.	1.0B
PARAKIEFFERIELLA SP.	2.0N
PARAMETRIOCNEMUS SP.	3.0H
PSECTROCLADIUS SP.	2.0H
RHEOCRICOTOPUS SP.	3.0H
SYNORTHOCLADIUS SP.	2.5N
THIENEMANNIELLA SP.	2.0H
THIENEMANNIELLA XENA	2.0H
TVETENIA SP.	2.0N
TVETENIA BAVARICA GROUP	2.0N
TVETENIA DISCOLORIPES GROUP	2.0N
CHIRONOMINAE	3.0N
CHIRONOMINI	3.0N
CHIRONOMUS SP.	5.0H
CHIRONOMUS DECORUS/RIPARIUS	5.0H
CHIRONOMUS FUMIDUS	5.0H
CHIRONOMUS REDUCTUS GR. (NR.)	5.0H
CHIRONOMUS RIPARIUS	5.0H
CHIRONOMUS TENTANS	5.0H
CLADOPELMA SP.	4.0N
CRYPTOCHIRONOMUS SP.	4.0H
CRYPTOCHIRONOMUS SP. 1	4.0H

TAXON NAME	TOLERANCE VALUE
CRYPTOCHIRONOMUS SP. 3	4.0H
CRYPTOCHIRONOMUS SP. C	4.0H
DICROTENDIPES SP.	4.0H
DICROTENDIPES/GLYPTOTENDIPES	4.0H
DICROTENDIPES MODESTUS	4.0H
DICROTENDIPES NEOMODESTUS	4.0H
DICROTENDIPES NERVOSUS	4.0H
DICROTENDIPES NERVOSUS TYPE 1	4.0H
DICROTENDIPES NERVOSUS TYPE 2	4.0H
EINFELDIA SP.	5.0H
ENDOCHIRONOMUS SP.	3.0H
GLYPTOTENDIPES SP.	5.0H
GLYPTOTENDIPES LOBIFERUS	5.0N
HARNISCHIA SP.	4.0H
HARNISCHIA SP. 2	4.0H
KIEFFERULUS SP.	4.0H
MICROTENDIPES SP.	3.0H
MICROTENDIPES CADUCUS	3.0H
MICROTENDIPES CAELUM	3.0H
MICROTENDIPES PEDELLUS	3.0H
MICROTENDIPES TARSALIS	3.0H
PARACHIRONOMUS SP.	4.0H
PARACHIRONOMUS ABORTIVUS	4.0H
PARACLADOPELMA SP.	3.0H
PARALAUTERBORNIELLA SP.	3.0H
PARATENDIPES SP.	2.0H
PARATENDIPES ALBIMANUS/DUPLICATUS	2.0H
PHAENOPSECTRA SP.	4.0H
PHAENOPSECTRA SP. 1	4.0H
PHAENOPSECTRA PROB. DYARI	4.0H
PHAENOPSECTRA FLAVIPES	4.0H
PHAENOPSECTRA OBEDIENS	4.0H
POLYPEDILUM SP.	3.0H
POLYPEDILUM AVICEPS	3.0H
POLYPEDILUM CONVICTUM	3.0H
POLYPEDILUM FALLAX GROUP	3.0H
POLYPEDILUM HALTERALE	3.0H
POLYPEDILUM ILLINOENSE	3.0H
POLYPEDILUM OBTUSUM	3.0H
POLYPEDILUM OPHIODES	3.0H
POLYPEDILUM SCALAENUM	3.0H
POLYPEDILUM SCALAENUM SP. 1	3.0H
POLYPEDILUM SCALAENUM SP. 2	3.0H
POLYPEDILUM SIMULANS/DIGITIFER	3.0H
POLYPEDILUM NR. TRIGONUM	3.0H
POLYPEDILUM TRITUM	3.0H
PSEUDOCHIRONOMUS SP.	3.0H
STENOCHIRONOMUS SP.	2.0H
STICTOCHIRONOMUS SP.	3.0H
STICTOCHIRONOMUS SP. 1	3.0H
STICTOCHIRONOMUS DIVINCTUS	3.0H
TRIBELOS SP.	1.0N
XENOCHIRONOMUS SP.	2.0H

TAXON NAME	TOLERANCE VALUE
XENOCHIRONOMUS (XENOCHIRONOMUS) SP.	2.0H
TANYTARSINI	3.0N
CLADOTANYTARSUS SP.	3.0H
CONSTEMPELLINA SP.	2.5N
MICROPSECTRA SP.	3.0H
PARATANYTARSUS SP.	3.0H
PARATANYTARSUS BOIEMICA	3.0H
PARATANYTARSUS DISSIMILIS	3.0H
PARATANYTARSUS DISSIMILIS/BOIEMICA	3.0H
RHEOTANYTARSUS SP.	3.0H
RHEOTANYTARSUS DISTINCTISSIMUS GROUP	3.0H
RHEOTANYTARSUS EXIGUUS GROUP	3.0H
STEMPELLINA SP.	2.0H
STEMPELLINA BAUSEI	2.0H
SUBLETTEA SP.	3.0H
SUBLETTEA COFFMANI	3.0H
TANYTARSUS SP.	3.0H
TANYTARSUS GLABRESCENS	3.0H
TANYTARSUS GLABRESCENS GROUP	3.0H
TANYTARSUS GUERULUS GROUP	3.0H
TABANIDAE (HORSE FLIES, DEER FLIES)	
CHRYSOPS SP.	3.0H
ATHERICIDAE	
ATHERIX SP.	2.0N
ATHERIX VARIEGATA	2.0H
DOLICOPODIDAE	2.0H
EMPIDIDAE	
CLINOCERA SP.	3.0H
HEMERODROMIA SP.	3.0H
EPHYDRIDAE	3.0H
BRACHYDEUTRA SP.	3.0H
SCATHOPHAGIDAE	2.0N
MUSCIDAE	2.0H
LIMNOPHORA SP.	2.0H
MOLLUSCA	
GASTROPODA (SNAILS)	
MESOGASTROPODA	
HYDROBIIDAE	
AMNICOLA SP.	
AMNICOLA LIMOSA	3.0N
BASOMATOPHORA	
LYMNAEIDAE	
LYMNAEA SP.	3.0N
PHYSIDAE	4.0N
PHYSA SP.	4.0N
PLANORBIDAE	
GYRAULUS SP.	4.0B
GYRAULUS PARVUS	4.0N
HELISOMA SP.	
HELISOMA ANCEPS	4.0N
HELISOMA CAMPANULATUM	4.0N
HELISOMA TRIVOLVIS	5.0N
ANCYLIDAE (LIMPETS)	

TAXON NAME	TOLERANCE VALUE
FERRISSIA SP.	3.0N
PELECYPODA (BIVALVES)	
HETERODONTA	
PISIDIIDAE (FINGERNAIL CLAMS)	3.0N

[1]Tolerance Value Sources:

B = R.W. Bode, Stream Monitoring Unit, New York State Department of Environmental Conservation, Albany, NY.

H = Hilsenhoff 1982.

J = Jones, et al. 1981.

N = R.M. Nuzzo, Technical Services Branch, Massachusetts Division of Water Pollution Control, Westborough, MA.

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2.3 SITE ASSESSMENT

2.3.1 INTRODUCTION AND PURPOSE

While site assessments make use of a number of qualitative and semi-quantitative methods borrowed from stream classification and/or rapid assessment protocols, they may also be expanded to include quantitative sampling and analytical procedures. In fact, site assessment surveys may range in scope from a qualitative assessment of the impact of a single wastewater discharge on a single aquatic community to intensive quantitative assessments of one or more communities. The latter are labor and resource intensive and are limited to those situations where the need exists for statistically derived statements of confidence in the results.

2.3.2 OBJECTIVES

1. To provide an adequate data base for making quantitative determinations of standing crop, biomass, or measures of community structure such as species diversity and richness;

2. to provide sufficient data for testing for significant differences between communities using appropriate statistical methods;

3. to provide standard methods for assessing the impacts of pollution on aquatic biota and water uses; and

4. to supplement physico-chemical water quality data with biological information.

2.3.3 APPROACH

Whenever possible, sampling stations are located upstream and downstream from known or suspected sources of pollution or other factors that might impact water quality conditions. The underlying assumption is made that, if all other environmental factors remain constant, a change in water chemistry will alter downstream community structure or biomass. Therefore, impact assessment is carried out by making community structural comparisons between upstream or nearby reference communities and downstream communities.

Measures of community structure to be employed are selected on a case-by-case basis according to the requirements of individual site assessments. Parameters include 1) abundance; 2) taxonomic richness; 3) evenness; and 4) diversity (e.g., Shannon Weaver H^1). Comparisons of communities between sites are made using the above measures and standard significance tests such as t-tests.

Less intensive site assessments involving the use of qualitative or semi-quantitative techniques are conducted according to the methods presented in previous sections for stream classification and rapid assessment surveys.

2.3.4 PARAMETRIC COVERAGE

Site assessments may involve the use of qualitative, semi-quantitative, or quantitative analyses of one or more of the following communities: phytoplankton; periphyton; macrophyton; macroinvertebrates; or fish. Biological stream sampling is supplemented, as deemed appropriate, by hydrological and physico-chemical assessments such as the determination of stream width, depth, flow, water temperature, substrate characterization, and chemical analyses.

2.3.5 QUANTITATIVE DATA ANALYSES

Definitions of some of the more commonly used indices of community structure are presented below.

Abundance

Two abundance measures are often used: (1) the sum total of individuals found in all taxonomic groups in a particular data set (termed "total numbers"); and (2) the relative proportion of individuals found in different taxonomic categories (termed "relative abundance").

If a relationship between productivity and numbers of individuals can be established, increases from control to test sites in the total number of organisms found may be a result of increased nutrient availability. Decreases in this measure may be related to changes in nutrients and/or the influence of toxic substances. Changes in the relative abundance of major taxonomic groups may be related to habitat alterations between sites. When changes in the relative abundance of major groups are accompanied by a decrease in richness (see below) they may be due to either changes in nutrient availability and/or to toxic stress.

Taxonomic Richness

This term refers to the number of different taxonomic groups in a particular sample. Comparisons of richness are based on the assumption that physiological stress (defined as those instances under which environmental conditions such as temperature, oxygen concentration, pH, etc., exceed the tolerance limits of an individual) due to a toxic discharge can reduce the number of taxa originally inhabiting a certain area.

Richness of a sample collection is positively correlated with sampling effort. As area sampled, time spent sampling, and/or number of organisms collected are increased, the number of different taxa encountered also increases. For these reasons, comparisons should only be made between data sets for which sampling efforts are similar or nearly so.

Evenness

This is a measure of the distribution of individual organisms over different taxonomic categories. Most evenness indices range from a value of zero to 1.0, with a completely uniform distribution yielding a value of 1.0.

Diversity indices (see below) compress richness and evenness into a single number. However, information is lost in this process. In an attempt to regain some of this information, ecologists have used evenness or equitability ratios that are usually of the form: measured diversity/ standard diversity, where the latter term is the maximum diversity of a community given a certain richness value. A basic problem with this approach is that the value or the ratio is dependent upon the particular characteristics of the diversity index. Thus, biases inherent to the index are incorporated into, and perhaps magnified by, the evenness ratio.

Diversity Indices

Most diversity indices attempt to interdigitate and refine two components of community structure: richness and evenness.

The Shannon Weaver H^1 is commonly used for two reasons: (1) it is simple in form; and (2) it has a known variance structure. Due to the latter attribute, a t-test for differences in H^1 between two data sets can be run. The form of the index and its variance structure are taken from Poole (1974) and are presented below.

$$H = \left(- \sum^{S} pi.\ln pi \right) - \frac{S-1}{2N}$$

$$\text{Var. } H' = \frac{\sum_{i=1}^{S} pi.\ln^2 pi - \left(\sum_{i=1}^{S} pi.\ln pi \right)^2}{N} + \frac{S-1}{2N^2}$$

where S = number of taxa
pi = the proportion of the total number of individuals consisting of the i^{th} taxon
N = total number of individuals

Another diversity index commonly used is Simpson's Index which can be defined as: D = 1 - C

$$\text{where } C = \sum_{i=1}^{S} \frac{ni(ni-1)}{N(N-1)}$$

and S = as above
ni = the number of individuals in the i^{th} species
N = as above

The term C is an approximation of the probability that two individuals drawn at random from a population of N individuals will belong to the same taxon. The higher this probability, the lower the "diversity" (as measured by this index) of the collection; hence D (equal to 1-C) is used as the index since this parameter will increase with the "diversity" of the sample.

The two indices cited above differ in their sensitivity to changes in richness and evenness. Whereas the Shannon Weaver Index is more an expression of the overall evenness of the community, the Simpson's Index expresses the relative degree of dominance of a few taxa in the community.

2.3.6 REFERENCES

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3.0 BIOLOGICAL FIELD AND LABORATORY METHODS

3.1 PHYTOPLANKTON

3.1.1 DEFINITION

DEFINITION: Phytoplankton are the algae of lakes and large rivers that live suspended in the water. They are chlorophyll-bearing, unicellular organisms which have no true roots, stems, or leaves. They occur in free-living, colonial, frond-like or filamentous forms and vary in size from unicells 0.5 microns in diameter to the macroscopic seaweeds. Algae are generally grouped into the Divisions (and classes) Euglenophyta (Euglenophyceae); Chlorophyta (Chlorophyceae, Charophyceae); Rhodophyta (Rhodophyceae); Cyanophyta (Myxophyceae); Pyrrophyta (Desmokantae, Dinophyceae); Chrysophyta (Xanthophyceae, Chrysophyceae, Bacillariophyceae); Phaeophyta (Phaeophyceae); and Cryptophyta (Cryptophyceae).

3.1.2 OBJECTIVES

1. To document the existing phytoplankton community and determine long-term (yearly) and short-term (seasonal) trends;

2. to evaluate direct effects on water composition including dissolved oxygen, pH, hardness, and optical properties;

3. to assess conditions affecting the general condition of water quality including noxious and toxic conditions, offensive tastes and odors;

4. to identify indicators of trophic status, organic enrichment and specific chemical contamination; and

5. to quantify autotrophic biomass and make inferences concerning productivity.

3.1.3 FIELD SAMPLING

Samples for phytoplankton analyses are collected in clean one-liter bottles made of plastic or glass, that have been rinsed with sample water. Approximately one-half liter of sample water is collected.

In rivers that are mixed vertically and horizontally, samples are collected midstream 0.5 to 1.0 meters (m) below the surface. In lakes and impoundments, samples are collected at the "deep-hole" station. If the lake is thermally unstratified the sample is collected 0.5-1.0 m below the surface. If the lake is thermally stratified, an integrated column sample is collected by lowering a one centimeter (approximately) ID plastic tube (with a weight attached) to the thermocline zone, pinched below the miniscus and raised into the boat. The sample is then drained into a clean and rinsed collection bottle. This procedure is repeated until one-half liter of water is collected. All samples are cooled to 4°C and placed in the dark following collection.

For special studies in riverine and lacustrine habitats, samples are collected from major depth zones or water masses. Sampling depths at each site are determined by specific conditions. In shallow areas (2-3 m), subsurface sampling is generally conducted. In deeper areas samples are collected at regular intervals at depths throughout the euphotic zone.

Pertinent information collected and recorded in the field includes meteorological data (cloud cover, wind speed and direction, air temperature); surface water conditions; water color, turbidity, odors; total depth at station; and other descriptive information.

The frequency of sampling is dependent on the intent of the study as well as the range of seasonal fluctuations, the immediate meteorological conditions, adequacy of equipment, and availability of personnel. In tidally-influenced habitats, phytoplankton samples are collected at all tide stages, particularly at the end and the beginning of both the flood and ebb tides.

3.1.4 LABORATORY ANALYSES

Sample Preservation

Phytoplankton samples collected in the field are cooled to 4°C and kept in the dark in transit to the laboratory. Upon arrival at the laboratory, they are placed in a refrigerator until further processing. Samples are generally analyzed on the day of collection. Samples not analyzed on the day of collection are stored in a refrigerator overnight with the caps loosened to allow gas exchange. Samples stored for more than 48 hours are fixed by the following methods and preservatives:

1. Lugol's solution: For short-term storage, 0.3 ml Lugol's solution is added per 100 ml of sample aliquot and stored in the dark. For long-term storage, 0.7 ml Lugol's solution is added per 100 ml of sample. [Lugol's solution is prepared by dissolving 20 grams (g) potassium iodide (KI) and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid].

2. Formalin: To preserve samples, 40 ml buffered formalin is added to one liter of sample.

3. M^3 Fixative: For preservation, 20 ml M^3 fixative is added to one liter of sample and stored in the dark. [M^3 is prepared by dissolving 5 g KI, 10 g iodine, 50 ml glacial acetic acid, and 250 ml formalin in one liter of distilled water].

Color -

Cupric sulfate solution is added to the sample to preserve color [Cupric solution is prepared by dissolving 21 g cupric sulfate in 100 ml distilled water].

Clumping -

To prevent clumping, a detergent solution is added to the sample [20 ml liquid detergent is added to 100 ml distilled water].

Phytoplankton Examination

Log-In Procedure -

1) Each sample is assigned a number and logged in as it is brought into the laboratory. The numbers are in consecutive order and are recorded both on the sample tag and in a notebook (log book).

2) Next to the number in the log book are also recorded the station number and location, date collected, date analyzed, initials of collector, type of samples, sample depth, and analyses requested, i.e., chlorophyll and/or algal identifications.

Phytoplankton Examination Equipment List -

1) Microscope - capable of 200x power with working distance greater than 1 mm.

2) Sedgwick-Rafter (S-R) counting cells

3) Whipple micrometer reticule

4) Stage micrometer

5) Pipettes

6) Bench sheets

7) Lens paper

Procedure for Filling the Sedgwick-Rafter Cell:

1) Place the cover glass diagonally across the cell.

2) Use large-bore 1 ml pipette to fill the S-R cell.

3) Place tip of the pipette in the corner of the S-R cell and slowly release the pressure of your finger on the end of the pipette. The cover slip will then rotate and cover the sample.

4) To reduce error:

   a. Do not overfill the cell which would yield a depth greater than 1 mm.

   b. Do not allow large air bubbles to form. To prevent the formation of these air spaces, a drop of distilled water is placed on the edge of the cover glass occasionally during the microscopic examination.

Procedure for Phytoplankton Examination:

1) Shake the sample bottle to mix well.

2) Rinse 1 ml pipette with distilled water (inside and out) and three times with sample water.

3) Fill counting cell with 1 ml of sample water (see: "Procedure for Filling the Sedgwick-Rafter Cell").

4) Allow sample to settle for 15 minutes (the settling rate for algae is 4 mm/hr; since the depth of the counting cell is 1 mm, a 15 minute settling time is used.

5) While sample is settling, prepare a microscopic slide or Palmer cell which will allow you to view the sample at a higher power. List the algal genera identified.

6) Use the keys to determine unknown organisms; particularly dominant ones.

7) Scan the Sedgwick-Rafter counting cell at 4x and determine need for concentration or dilution.

8) At 200x find the edge of the counting cell and focus on the top of the cell. Continue turning the coarse focusing knob on the microscope until the bottom of the cell comes into focus.

9) At least two strips in the S-R counting cell must be counted.

10) Counts are done on both the bottom of the cell and the top or underside of the cover slip.

11) Identify and count all the algae that are located in the Whipple grid. Algae which are half in and half out of the top of the grid should be included in the count. Algae which are half in and half out of the bottom of the grid are not included in the count.

12) If the algal density appears to be high then fields can be counted instead of strips. A field is represented by a Whipple grid. Ten fields on two slides are counted and then averaged.

13) A strip is represented by the width of Whipple grid and the length of a Sedgwick-Rafter cell.

Explanation of the Phytoplankton Examination Sheet:

(Refer to: "Phytoplankton Examination" Form)

1) Line 1 - station location, station number, date of collection

2) Line 2 - initials of analyst, milliliters of sample, which will be either 1 ml or the total concentrated, type of count, i.e., fields or strips and the date of analysis.

3) Lab number - the number assigned the sample by the investigating laboratory (see: "Log-In Procedures").

4) Bottom two lines - chlorophyll in mg/m^3, total live algae (cells/ml), multiplication factor (S-R) for the particular microscope and power used, microscope manufacturer and type, the microscope power used (10x, 20x, etc.), type of preservative used, and a box for the initials of the person who does the quality control check of the multiplication and addition on the examination sheet.

5) Center of the phytoplankton examination sheet - seven algal classes and eight types are delineated. Identifications are recorded under the organism column, running counts are recorded under counts. The running counts are tallied and multiplied by the S-R factor to obtain totals in cells/ml. A total is given for each class and type as well as for the sample.

Determination of the S-R Factor:

When strip counts or field counts are done on a Sedgwick-Rafter counting cell, only a portion of the 1 ml sample is examined. Therefore, a calibration of "S-R" factor must be determined. The following formula is used in this calibration:

$$\text{S-R factor (strip count)} = \frac{1000}{\text{LxWxDxS}}\ mm^3$$

where: L = length of a strip (mm)
S-R cell is 500 mm long

W = width of a strip which is the Whipple grid image width (determined by using a stage micrometer)

D = depth of chamber (1 mm)

S = number of strips counted

The S-R (strip count) times C, the number of organisms counted (tally) equals the number of algae per milliliter.

$$\text{units/ml} = \text{S-R (strip count)} \times C$$

The S-R factor (field count) is calculated by using the following formula:

$$\text{S-R factor (field count)} = \frac{1000}{\text{AxDxF}}\ mm^3$$

where: A = area of a field, which is the Whipple grid image area

D = depth of chamber (1 mm)

F = number of field counts

The number of algae per ml equals the S-R (field count) times C, the number of organisms counted (tally).

Units/ml = S-R (field count) x C

Procedure for Phytoplankton Counts:

In the unit (or clump) count each cell or colonial group of cells receives one unit.

Examples:

1. Anacystis - one unit per clump

2. Anabaena - one unit per chain

3. "Filamentous green" - one unit per filament

4. Scenedesmus - one unit each (4, 8, 16 etc., celled organism.)

5. Fragilaria and Melosira - count each cell (may be best to average the area for a single cell and divide into total area.)

6. Asterionella - each "arm" one unit

7. Dinobryon - each colony one unit.

An attempt is made to identify all organisms to generic level. If this can not be accomplished then an effort is made to assign the organism to the proper class and type. Unidentified organisms are described as "UI" on the phytoplankton examination sheet. Subscripts are assigned, i.e., "UI1", "UI2", "UI3", etc., if more than one kind of unidentified organism are present within a particular class and type.

Counts below 500 cells/ml are generally unreliable. In general, an attempt is made to observe at least 20 organisms while making tallies in strip counts. Any manipulation of the sample (concentration or dilution) adds error. Therefore, on samples with high concentrations, a field count rather than concentrate is performed. On samples with low counts, more strips are counted. Precision is achieved in field counts by determining the coefficient of variation for counts in the number of fields counted and adjusting the number of fields counted to meet an $\pm$ 10% error, as outlined in precision calculations (see: "Precision Data").

Precision Data:

1. $S = \sqrt{\frac{\sum(x-m)^2}{n-1}}$

   Where:

   S = Standard deviation
   M = Mean (average)
   X = Count
   n = Number of fields

2. $C_v = \frac{s}{m}$ or $\sqrt{\frac{\sum_x^2}{M^2} - n}{n-1}$

   Where C_v = Coefficient of variation

3. P = % standard deviation of mean = $\frac{100c_v}{\sqrt{n}}$

4. C_v must be 0.317 or less if results in a 10-field count are to be $\pm$ 10% within a 2/3 probability and a practical certainty (95%) of $\pm$ 20% precision error.

5. Using past data it was found that if ten fields are counted:

   C_v = 1.0 or $\pm$ 31.7% error was found in 90% of samples
   0.7 or $\pm$ 22% error was found in 75% of samples
   0.45 or $\pm$ 14% error was found in 50% of samples
   0.317 or $\pm$ 10% error was found in 33% of samples

   On the average, a third of random samples were within $\pm$10% error when 10 fields were counted, and half were within $\pm$14%.

6. P = The Standard error of count (percent) and is found in the log-log plot of C_v versus n.

3.1.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat, motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ plastic tubing with weight attached
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ sample preservative, fixative

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.1.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

PHYTOPLANKTON FIELD DATA RECORD

RIVER:

SITE:

STATION(S):

COLLECTOR(S):

DATE:

SAMPLE NUMBER(S):

LABORATORY NUMBER(S):

TYPE OF SAMPLE:

Sample Depth(S):

Water Temperature

Light Attenuation:

Secchi Disk

Illuminance Meter

Surface Foot-Candles

Foot-Candles

SAMPLE EQUIPMENT/METHODS:

REMARKS:

SITE MAP

HABITAT DESCRIPTION

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TECHNICAL SERVICES BRANCH

PHYTOPLANKTON EXAMINATION SHEET

River/Lake		Station	Date Collected	Lab No.
Analysis by:	Mi	Count	Date Analyzed	

Class	Type	Organism	Count	Tally	Cells/ml	Total
Bacillariophyceae (Diatoms)	Centric					
	Pennate					
Cyanophyceae (Blue-Greens)	Coccoid					
	Filamentous					
Chlorophyceae (Greens)	Coccoid					
	Desmids					
	Filamentous					
	Flagellates					
Chrysophyceae (Golden-Browns)						
Cryptophyceae (Cryptamonads)						
Dinophyceae (Dinoflagellates)						
Euglenophyceae (Euglenids)						

Chlorophyll a / in mg/m^3 Tot. live algae (c/ml) SR =

Microscope _____ Power _____ Preserved _____ Quality Control

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3.2 PERIPHYTON

3.2.1 DEFINITION:

Periphyton as used here shall mean the attached algal community. Any associated bacteria, fungi, mosses or epiphytic animals are identified to a rudimentary level only. Occasionally, planktonic algae are collected during periphyton sampling in lotic waters. These identifications are reported under the heading periphyton, although, strictly speaking, they are plankton.

3.2.2 OBJECTIVES

1. To document the existing periphyton component in lotic and lentic environments and determine dominant types; and

2. to evaluate water quality conditions by the use of indicator species.

3.2.3 FIELD SAMPLING

Prior to disturbing the streambed or lakebed, stations (or reaches) selected for qualitative investigation are visually inspected for algal growth. Representative samples are collected from each macrohabitat: pools, riffles, channel, streambank, backwater, open-water; and all substrates: rocks, sand, vegetation, twigs and other debris. Each type of alga encountered is collected using forceps, pipette, knife or by hand. Specimens are placed in labeled glass (or plastic) vials with water from the sampling site, and deposited into a cooler on ice for transportation to the laboratory. Information concerning growth habit and relative abundance of the representative algae are duly noted on field sheets. Photographic documentation of site conditions may also be conducted.

3.2.4 LABORATORY ANALYSES

Log-In Procedure

Each sample is recorded in the algae log book along with the phytoplankton samples. The sample is assigned a number followed by the letter P indicating periphyton. This is done in order to distinguish it from phytoplankton samples. Also recorded in the log book are the station number and location, the date collected, initials of the collector, and date analyzed.

Microscopic Analysis

Samples collected in the field are stored in the refrigerator until they are viewed. Specimens are identified within one to two days following collection while they are still alive and healthy. This facilitates identification since preservatives tend to alter the color and - in some cases - the structure of the algae. Identifications are made from wet mounts using a compound microscope equipped with 10x, 20x, 40x, and 100x objectives. Identifications are made using various taxonomic keys to the lowest level possible and recorded on a Periphyton Lab Bench Sheet (see Section 3.2.6). Certain specimens are photographed as a means of documentation and for use in presentations or as a teaching tool. Taxonomic lists of the results are compiled for each survey and published in appropriate reports.

Periphyton Examination Laboratory Equipment List

1. Microscope with 10x, 20x, 40x, 100x objectives
2. Microscope slides and coverslips
3. Pipettes
4. Forceps, probes
5. Lens paper
6. Bench sheets

3.2.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ sample preservative, fixative

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.2.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

PERIPHYTON FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION:	SAMPLE NUMBER(S):

HABITAT	LIGHT	WATER TEMPERATURE	SAMPLE EQUIPMENT/METHODS:
Pool Riffle Backwater Impoundment Other	Open Total Shade Partial Shade Comments:		

Sample #	Substrate Type	Areal Cover(%)	Growth Habit	Color	Texture	Smell

Site Map:	Other Remarks:

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TECHNICAL SERVICES BRANCH

PERIPHYTON LAB BENCH SHEET

BASIN: _____ BASIN NUMBER: _____

RIVER: _____ STREAM INVENTORY NUMBER: _____ TOWN: _____

STATION: _____ COMMENTS: _____

Date Collected: _____	Date Analyzed: _____
Collector(s): _____	Analysis by: _____
_____	Microscope: _____ Power: _____
Number of Samples: _____	Photo: _____

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Relative Abundance: Most Abundant, Abundant, Common, Sparse
Habitat: Pool, Riffle, Backwater, Impoundment, Spillway, etc.
Substrate: Rock, Mud, Sand, Wood, Bottle, etc.

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3.3 AQUATIC AND WETLAND VEGETATION

3.3.1 DEFINITION

DEFINITION: Aquatic flora as used here pertains to several taxonomic groups including the Characeae (stoneworts and muskgrass); Musci, Hepaticae, and Ricciaceae (mosses, leafy liverworts, thallose liverworts); Osmundaceae (flowering ferns); Equisetaceae (horsetail, scouring rush); Isoetaceae (Quillwort); and the Angiospermae (the seed plants).

3.3.2 OBJECTIVES

1. To identify and test reliable methods and procedures for the collection, identification and enumeration of aquatic and wetland vegetation;

2. to document existing aquatic plant species and communities; and

3. to determine areal coverage and dominant plant types.

3.3.3 FIELD SAMPLING

For riverine habitats, aquatic and wetland vegetation are located and qualitatively mapped by visually examining the streambed, streamside, and immediate riparian areas by walking or wading. A reach of stream approximately 10-meters in length is generally investigated. Each macrohabitat is sampled and the predominant vegetation noted and recorded on standard type field data sheets. A schematic map is prepared for each site. Photographic documentation is sometimes made. Vegetation is generally identified on-site.

The aquatic and wetland plant community in lacustrine habitats is located and mapped by examining the limnetic, shoreline, and littoral areas by boat or waders. Occasional samples are collected at regular intervals on imaginary transects run across open-water areas of the lake or impoundment. All habitats are sampled and the relative abundance of each plant type noted and mapped on prepared outline maps. Representative macrophytes are collected by hand and, in deeper water, by dragging a simple grappling hook with a weight attached to the shaft. An Ekman or Ponar dredge is sometimes used to collect deeply-submerged vegetation. Identifications of most plant specimens are made in the field.

3.3.4 LABORATORY ANALYSES

Vegetation not identified in the field is collected and returned to the laboratory for further analysis using a stereoscopic microscope or hand lens and various taxonomic keys. Representative plant specimens collected from each site are pressed and dried in preparation for permanent mounting. Plant specimens are deposited in the Botanical Reference Library of the Technical Services Branch.

3.3.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can, (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ plastic bags (and ties)
- ☐ sample preservative, fixative
- ☐ rake
- ☐ grappling hook, rope
- ☐ Ekman, Ponar dredges
- ☐ white enamel trays
- ☐ trowel
- ☐ plant press and vasculum

Field Apparel

- ☐ rain gear, (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.3.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC VASCULAR PLANT FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION(S):	SAMPLE NUMBER(S):

TAXA	DEPTH	AREAL COVER(%)	REMARKS	SITE MAP

SAMPLING EQUIPMENT/METHODS

OTHER REMARKS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM-SIDE AND RIPARIAN HABITAT FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION(S):	SAMPLE NUMBER(S):

STREAM-BANK/SHORELINE CHARACTER:	REMARKS:

FLOODPLAIN

Structural	Areal Cover(%)	Composition/Type
1. Trees		
2. Shrubs		
3. Herbaceous		
4. Grasses		
5. Others		
6. Open		

SITE MAP

UPLAND

SAMPLE EQUIPMENT/METHODS

OTHER REMARKS:

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC MACROPHYTE CODE

Macroscopic algae (mats, clumps, etc.)	
Bryozoan	
Chara sp. "Muskgrass"	C1
Nitella sp. "Stonewort"	C2
Moss	
Riccia fluitans "Slender Riccia"	
Ricciocarpus natans "Purple-fringed Riccia"	
Osmunda regalis "Royal Fern"	
Marsilea quadrifolia "Pepperwort"	
Azolla caroliniana "Water-velvet"	
Salvinia rotundifolia "Floating Moss"	
Other aquatic ferns	
Isoetes sp. "Quillwort"	I
I. Tuckermani "Quillwort"	I1
Typha latifolia "Common Cattail"	T
Typha angustifolia "Narrow-leaved Cattail"	T1
Sparganium sp. "Bur Reed"	S
S. fluctuans "Water Bur Reed"	S1
S. eurycarpum "Giant Bur Reed"	S2
S. americanum "Bur Reed"	S3
Potamogeton sp. "Pondweed"	P
P. amplifolias "Largeleaf Pondweed"	P1
P. crispus "Curlyleaf Pondweed"	P2
P. Richardsonii "Richardson Pondweed"	P3

P. Robbinsii "Flatleaf Pondweed"	P4
P. epihydrus "Ribbonleaf Pondweed"	P5
P. sp. "Thin-leaved Pondweed"	P6
P. gramineus "Grassleaf Pondweed"	P7
P. natans "Floatingleaf Pondweed"	P8
P. Vaseyi "Vasey's Pondweed"	P9
P. capillaceus "Pondweed"	P10
P. foliosus "Leafy Pondweed"	P11
P. tenuifolius "Pondweed"	P12
P. perfoliatus "Redhead Grass" or "Thorowort Pondweed"	P13
P. pusillus "Slender Pondweed" or "Baby Pondweed"	P14
P. Spirillus "Snailseed Pondweed"	P15
P. pectinatus "Sago Pondweed" or "Fennelleaf Pondweed"	P16
P. illinoensis "Illinois Pondweed"	P17
P. pulcher "Heartleaf Pondweed"	P18
P. bicupulatus "Snailseed Pondweed"	P19
P. zosteriformis "Flatstem Pondweed"	P20
P. nodosus	P21
Najas sp. "Bushy Pondweed" or "Naiad"	J
Ruppia maritima "Widgeon Grass"	J1
Najas Flexilis "Slender Naiad"	J2
Alisma sp. "Water-Plantain"	A1
Echinodorus sp. "Burhead"	A2
Sagittaria sp. "Arrowhead" or "Duck Potato"	A3
Sagittaria sp. (submerged form only)	A4
S. latifolia "Common Arrowhead"	A5
S. rigida "Stiff Arrowhead"	A6
S. teres "Dwarf Wapato"	A7
S. graminea "Grassy Arrowhead"	A8
Vallisneria americana "Wild Celery" or "Tape Grass"	H1
Elodea sp. "Waterweed"	H2
E. Nuttallii "Waterweed"	H3
E. canadensis "Canadian Waterweed"	H4
Gramineae (Grass Family)	G

Cyperus sp. "Flat Sedge"	Y1
Dulichium arundinaceum "Three-way Sedge"	Y2
Fimbristylis sp. "Fimbristylis"	Y3
Rynchospora sp. "Beak Rush"	Y4
Cladium sp. "Twig Rush" or "Sawgrass"	Y5
Carex sp.	X
Scirpus sp. "Bulrush"	B
S. validus "Softstem Bulrush"	B1
S. cyperinus "Woolgrass Bulrush"	B2
S. americanus "American Bulrush"	B3
S. atrovirens "Dark-green Bulrush"	B4
Eleocharis sp. "Spike Rush"	E
E. acicularis "Needle Spike Rush"	E1
E. Smallii "Spike Rush"	E2
E. palustris "Common Spike Rush"	E3

Peltandra virginica "Arrow Arum"	a1
Calla palustris "Water Arum"	a2
Orontium aquaticum "Golden Club"	a3
Acorus Calamus "Sweet Flag"	a4
Spirodela polyrhiza "Big Duckweed"	L1
Wolffia sp. "Watermeal"	L2
Wolffiella floridana "Florida Wolffiella"	L3
Lemna sp. "Duckweed"	L4
L. minor "Common Duckweed"	L5
L. trisulca "Star Duckweed"	L6
Xyris sp. "Yellow-eyed Grass"	e
Eriocaulon sp. "Pipewort"	e1
E. septangulare "Pipewort"	e2
Heteranthera dubia "Mud Plantain"	W1
Pontederia cordata "Pickerelweed"	W2
P. cordata forma *taenia* "Pickerelweed"	W3

Iris sp. "Iris"	j1
Juncus sp. "Rush"	j2
Soururus cernuus "Lizard's Tail"	j3
Rumex sp. "Dock"	Q1
Polygonum sp. "Smartweed"	Q2
Salix sp. "Willow"	b1
Myrica Gale "Sweet Gale"	b2
Alnus sp. "Alder"	b3
Nyssa sp. "Sour Gum" or "Tupelo"	b4
Cornus sp. "Dogwood"	b5
Chamaedaphne calyculata "Leatherleaf"	b6
Fraxinus sp. "Ash"	b7
Cephalanthus occidentalis "Buttonbush"	b8
Ilex verticillata "Virginia Winterberry" or "Black Alder"	b9
Clethra alnifolia "Sweet Pepperbush"	b10
Ceratophyllum demersum "Coontail"	K
Nymphaea sp. "Water Lily"	N1
N. odorata "Fragrant Water Lily"	N2
N. tuberosa "White Water Lily"	N3
Nuphar sp. "Yellow Water Lily", "Cow Lily", or "Spatterdock"	N5
N. variegatum "Painted Cow Lily"	N6
Brasenia Schreberi "Water Shield"	n1
Nelumba lutea "American Lotus"	n2
Cabomba caroliniana "Fanwort"	n3
Caltha palustris "Marsh Marigold"	R1
Myosurus minimus "Mousetail"	R2
Ranunculus sp. "Buttercup" or "Crowfoot"	R3
Subularia aquatica "Awlwort"	M1
Neobeckia aquatica "Lake Cress"	M2
Cardamine sp. "Bitter Cress"	M3
Rorippa sp. "Water Cress"	M4

Podostenum sp. "River Weed"	r
Callitriche sp. "Water Starwort"	k1
Elatine sp. "Waterwort"	k2
Viola sp. "Violet"	k3
Hypericum sp. "St. John's-wort"	k4
H. boreale f. callitrichoides "St. John's-wort"	k5
Decodon verticillatus "Swamp Loosestrife"	V1
Trapa natans "Water Chestnut"	V2
Ludwigia sp. "False Loosestrife"	V3
Lythrum Salicaria "Spiked Loosestrife"	V4
Rhexia virginica "Virginia Meadow-beauty"	V5
Hippuris vulgaris "Mare's-tail"	h1
Prosperinaca sp. "Mermaid Weed"	h2
Myriophyllum sp. "Water Milfoil"	h3
M. heterophyllum "Broadleaf Water Milfoil"	h4
M. humile "Water Milfoil"	h5
M. tenellum "Leafless Milfoil"	h6
Sium suave "Water Parsnip"	f1
Hydrocotyle sp. "Water Pennywort"	f2
Cicuta sp. "Water Hemlock"	f3
Hottonia inflata "Featherfoil"	m1
Samolus sp. "Water Pimpernel"	m2
Lysimachia sp. "Loosestrife"	m3
L. ciliata "Loosestrife"	m4
Nymphoides cordatum "Floating Heart"	g1
Asclepias sp. "Milkweed"	g2
Myosotis sp. "Forget-me-not"	g3
Stachys sp. "Hedge Nettle"	t1
Scutellaria sp. "Skullcap"	t2
Physostegia sp. "False Dragonhead"	t3
Lycopus sp. "Water Horehound"	t4

Mentha sp. "Mint"	t5
Solanum Dulcamara "Nightshade"	t6
Utricularia sp. "Bladderwort"	U
U. vulgaris "Common Bladderwort"	U1
U. purpurea "Purple Bladderwort"	U2
U. inflata "Floating Bladderwort"	U3
U. intermedia "Flat-leaved Bladderwort"	U4
Bacopa sp. "Water Hyssop"	F1
Limosella sp. "Mudwort"	F2
Veronica sp. "Speedwell"	F3
Chelone sp. "Turtlehead"	F4
Mimulus sp. "Monkey Flower"	F5
Lindernia sp. "False Pimpernel"	F6
Gratiola sp. "Hedge Hyssop"	F7
G. virginiana "Hedge Hyssop"	F8
Lobelia sp.	O
L. cardinalis "Cardinal Flower"	O1
L. Dortmanna "Water Lobelia"	O2
Megalodonta Beckii "Water Marigold"	Z1
Eupatorium sp. "Joe-pye Weed"	Z2
Bidens sp. "Bur Marigold", "Beggar-ticks", or "Pitchforks"	Z3
Helenium sp. "Sneezeweed"	Z4
Solidago sp. "Goldenrod"	Z5
Aster sp. "Aster"	Z6
Coreopis rosea "Pink Tickseed"	Z7
Equisetum sp. "Horsetail"	i
E. fluviatile "Swamp or Water Horsetail"	i1
Drosera rotundifolia "Roundleaf Sundew"	D
Vaccinium sp. "Cranberry"	d
Phragmites sp. "Reed Grass"	q

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3.4 AQUATIC MACROINVERTEBRATES

3.4.1 DEFINITION: The aquatic macroinvertebrate community is defined as the assemblage of invertebrate organisms which can be seen by the unaided eye and retained by a U.S. Standard No. 30 sieve (i.e., 28 meshes per inch; 0.595 mm apertures). All or some life-cycle stages of these animals occur either attached to plants, other animals, debris, or inorganic substrates, or they float or swim in the water column of lentic and lotic waterbodies. Representative members of this community include - but are not limited to - sponges, bryozoa, flat worms, segmented worms, arthropods (water mites, crustaceans, insects), and mollusks.

3.4.2 OBJECTIVES

1. To provide information for stream classification, assessment of water quality conditions and trends, and direct impact assessment;

2. to interpret data using knowledge of the pollution ecology of component taxa (e.g., indicator schemes; biotic indices), or by observing changes in invertebrate community structure (e.g., richness; diversity); and

3. to determine the severity of water pollution problems by comparing unimpacted control or reference communities with potentially impacted communities.

3.4.3 FIELD SAMPLING

Qualitative

Qualitative macroinvertebrate sampling for stream classifications or special site assessments involves the use of a variety of sampling devices to collect samples from all available habitats encountered within a sampling site. Generally, D-frame nets are used to sweep aquatic vegetation, collect under cut stream banks, and agitate substrates to dislodge benthic organisms. Depending upon the taxonomic level desired, organisms are identified in the field to family level or placed in jars with 70% ethanol (95% if sample contains sediment materials or debris) for transport to the laboratory where further analyses are conducted.

Rapid Assessment

To obtain a sample for the Macroinvertebrate Rapid Bioassessment (MRB), a D-frame net is pressed against the substrates, and substrate material just upstream and in front of the net is agitated by kicking. This procedure is continued for five minutes while gradually moving upstream. Sampling is executed in areas of comparable substrate and current velocity (usually riffle areas within the central one third of the channel).

At the end of the five minutes of kick-sampling the contents of the net are emptied into a white enamel pan. Organisms clinging to the net are removed, using forceps, and placed in the sample container, as are

organisms on substrate materials too large to fit into the sample container. Once the organisms have been removed, these larger materials are returned to the stream. The remainder of the sample is added to the container and preserved with 95% ethanol, containing 130 mg/l Rose Bengal stain. Completed labels are placed inside each container and attached to the outside. Field notes record the major taxonomic groups encountered during field processing.

Quantitative

When quantitative sampling is required, the following routine is employed:

1. Depending on depth, flow, and substrate conditions sampling gear is selected from among Ekman, Petersen and Ponar grab samplers or Surber and Hess substrate samplers. One set of four replicate samples is obtained following a random transect whereby both banks and two quarter points are sampled.

2. The substrate obtained is characterized according to particle size and composition, placed into a basin, and mixed thoroughly. When the sample consists of heavily organic or sand-silt type substrate, one-quarter of the sample is randomly selected and retained after mixing. The remaining material is qualitatively examined and discarded. Subsampling is often necessary due to the time required for sorting a large quantity of substrate.

3. The sample portion is passed through a standard U.S. No. 30 brass sieve (0.595 mm apertures). Organisms and substrate left behind are placed into labeled plastic or glass wide-mouth containers (approx. 1 liter) and returned alive or preserved with 95% ethanol to the laboratory for further analysis.

3.4.4 LABORATORY ANALYSES

All samples are recorded in a log book upon arrival at the laboratory. Preserved samples are drained on a U.S. Standard No. 30 mesh screen and rinsed with tap water. Live and preserved samples are placed in individual white enamel pans for sorting. Samples for quantitative analyses are preferably sorted alive by removing all benthic organisms manually from the substrate and separating them by taxonomic order into glass vials containing 70% ethanol. For the MRB the contents of the enamel pan are subdivided by scooping material successively (one after the other) into four to eight glass petri dishes until all the material is distributed among the dishes. The number of dishes used depends on the volume of substrate and debris in the sample.

Before picking out organisms, the petri dishes are assigned a number (one to four, if four are used). Numbers are then drawn at random to determine the order of processing. The dish with the number corresponding to the first number drawn is placed on the stage of a stereomicroscope without deliberate orientation (first random field). All organisms within the field of view at low power are picked and placed in labeled vials with 70% ethanol. When all organisms in the field of view have been removed

the dish is moved to another random field for removal of additional organisms. This procedure is repeated until 100 organisms have been selected, moving to the next randomly selected petri dish as required. The remaining sample materials are again sieved on a #30 mesh screen, labeled, and archived in 95% ethanol. Extracted specimens are stored in 70% ethanol.

Macroinvertebrate specimens other than chironomids and oligochaetes are identified through examinations using a Wild M5A stereomicroscope equipped with fiber optics lighting. Oligochaetes, chironomid larvae, and chironomid pupae must be mounted on microscope slides before examination with an Olympus BH-2 compound microscope equipped with Nomarski optics. Each vial of specimens and each slide of mounted material must be labeled as described under "Instructions For Labeling Specimens."

3.4.5 INSTRUCTIONS FOR MOUNTING MIDGE LARVAE AND PUPAE

INSTRUCTIONS FOR MOUNTING MIDGE LARVAE

1) Place larvae in a watch glass along with a sufficient volume of 70% alcohol to keep them covered;

2) Under low power on a dissecting scope, group the larvae according to size and general appearance. This usually results in midges of the same tribe or subfamily being mounted together, which in turn will aid in working specimens through the keys more quickly;

3) Place a label on one end of a 25 x 75 mm glass microscope slide. With the label to the right, place three similar-sized larvae together on the slide, all with their heads pointed down;

4) Add enough CMC-10 to surround the specimens. Spread out the mounting medium so it doesn't sit in a "bubble" around the specimens;

5) Excise the head capsules, making sure that each remains associated with its own body, and is positioned with the mouth parts (ventral side) up;

6) Place a glass cover slip (No. 1, 18 mm x 18 mm) over the specimens and, using forceps, apply gentle pressure to force out air bubbles and flatten the head capsules until the mouth parts spread out slightly;

7) Add additional mounting medium to the edge of the cover slip if necessary to rid the mount of air bubbles;

8) Record any useful observations about the specimens (e.g., if ventral tubules are present, and how many pairs) on the SLIDE INVENTORY CATALOG SHEET. Refer to the specimens based on their position relative to the label: "A" indicating the cover slip closest to the label and "1" indicating the specimen closest to the label. Thus, A1 is the specimen closest to the label and B3 is the furthest (see fig. 1).

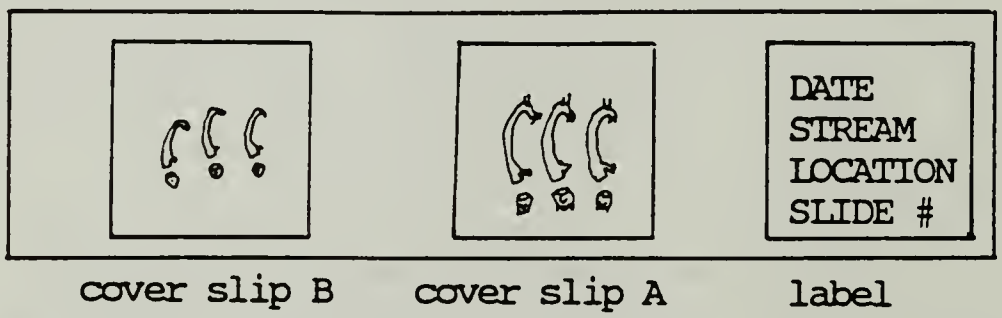

Figure 1. Layout of midge larvae mounted on a glass microscope slide.

9) Allow the mount to dry overnight before putting the slides into a slide box. Also check for air bubbles creeping under the edges of the cover slips as the medium dries. Keep the SLIDE INVENTORY CATALOG SHEET with the slide box so that the notes on the specimens are handy while keying out the organisms, and for recording the identifications.

INSTRUCTIONS FOR MOUNTING MIDGE PUPAE

1) Place midge pupae in watch glass along with 70% ethanol and examine under a dissecting microscope;

2) Record notes of markings, the form of the thoracic horn (if present), and the form of the case (if any) around the pupa (Rheotanytarsus spp. larvae and pupae can be recognized quickly from the case);

3) If the larval skin and head capsule are not still attached to the pupal body the pupa should be cleared before mounting (if larval skin, with larval head intact, is present, skip to next step). Clear the pupa by "cracking" the dorsal thoracic suture with a fine insect pin (0 or 00) and immersing the specimen in 10% KOH for two hours (if there is more than one pupal form present, clear each in a separate vial). After the two hours rinse the pupa in distilled water and place in 70% ethanol;

4) With the labeled end of a 25 x 75 mm slide to the right, place a pupa on the slide with ventral side down, with its head pointing toward the bottom edge of the slide. Cover specimen with CMC-10 mounting medium. Using an insect pin, carefully detach the head from the body, and position the head ventral side up. If the larval head capsule is included, mount it next to the pupa with the ventral side up. Spread the wing sheaths out to the sides of the thorax. Drop cover slip into place and gently force out air bubbles, but avoid "squashing" the pupa anymore than absolutely necessary. Mount only one individual per cover slip, but two cover slips per slide (see fig. 2);

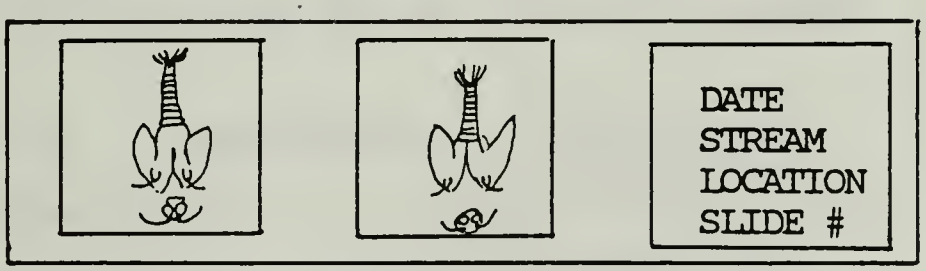

Figure 2. Layout of midge pupae mounted on a glass microscope slide.

5) Apply additional mounting medium to the edges of the cover slips if required to remove air bubbles. After 24 hours of drying examine the mount for air under the cover slips caused by shrinkage of the mounting medium, add mounting medium as required.

6) Be sure the record notes about specimen directly onto the SLIDE INVENTORY CATALOG SHEET.

3.4.6 INSTRUCTIONS FOR LABELING SPECIMENS

1) All vials and slides containing macroinvertebrate specimens from TSB biomonitoring surveys will be labeled immediately;

2) Each label will contain the following minimum information:
   a) biomonitoring tracking number
   b) station code
   c) date of collection
   d) name of stream organism was collected from
   e) location (town, state, nearest road);

3) Additionally, each vial label will list the name of the organisms contained inside the vial, whereas the labels placed on slides will show the number of the slot it occupies in the slide storage box;

4) Labels for use in vials will be cut to an appropriate size from unlined index cards. These will be placed inside the vial, and therefore must be written out with pencil or black India ink. As a convenience for locating and reading information, all the information listed in 2 above will be shown on one side of the label, and the left edge of the label will be placed in the vial first. The sequence for filling in information on the vial label is shown in the example in figure 3;

5) Stick-on labels will be used for microscope slides. Information will be written on the label such tht it appears right side up when the slide is in place on the stge of the microscope with the label to the right-as shown in the example in figure 4.

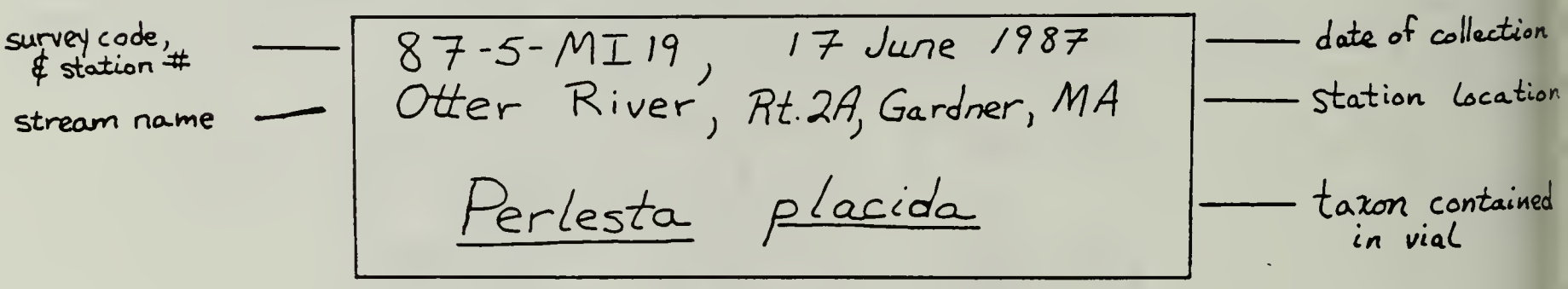

Figure 3. Example of specimen label to be placed inside a vial.

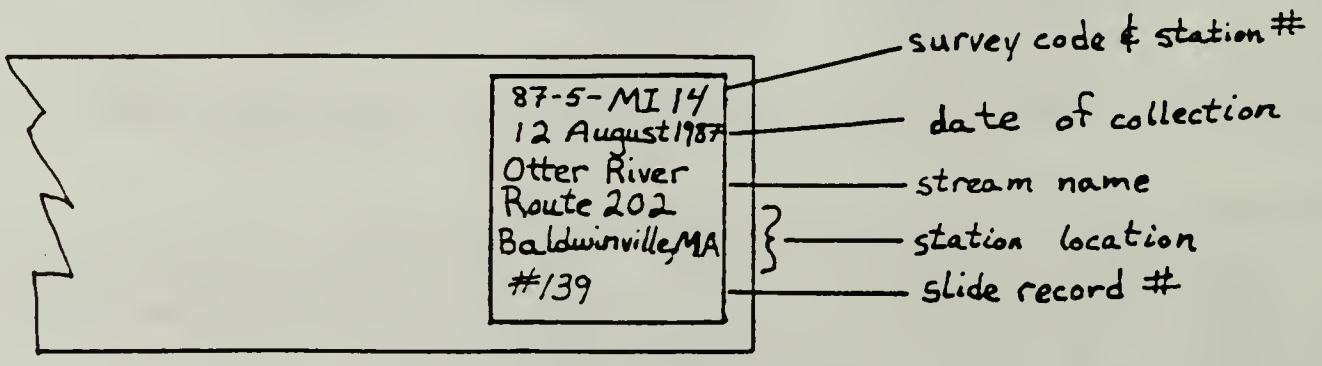

Figure 4. Example of label to be placed on microscope slide mount.

3.4.7 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ pocket thermometer
- ☐ tape measure
- ☐ range finder
- ☐ Ekman, Peterson, Ponar dredges
- ☐ Surber samplers
- ☐ Hess sampler
- ☐ metal holding tub
- ☐ white enamel trays
- ☐ sieves (of various sizes)
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ ethanol, formalin
- ☐ killing jar, killing agent
- ☐ aerial net, D-frame net

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.4.8 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC MACROINVERTEBRATE FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
SAMPLE NUMBER(S):	SAMPLING DURATION:

HABITAT:					
BOTTOM TYPE:					
BOTTOM VEGETATION:					
SAMPLE DEPTH:					
DOMINANT ORGANISM/ASSEMBLAGE:					
SAMPLE EQUIPMENT/METHODS:					

SITE MAP:	OTHER REMARKS:

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC MACROINVERTEBRATE LAB BENCH SHEET

Name of Water Body_____ Station No._____ Location_____
Date Collected_____ Collector_____ Sorted By_____ Code_____

ORGANISM	# /LS/TV/TI	ORGANISM	# /LS/TV/TI
Nematoda		Plecoptera	
Annelida		.....	
Oligochaeta		Hemiptera	
Hirudinea		.....	
		Megaloptera	
Isopoda			
		.....	
Amphipoda		Trichoptera	
Decapoda		.....	
		Coleoptera	
Hydracarina			
		.....	
		Diptera	
Collembola			
.....			
Ephemeroptera			
		Gastropoda	
		Pelecypoda	
.....			
Odonata		Others	

Total No. of Organisms_____
Total No. of Kinds_____

\# = Number of individuals tallied
TV = Biotic Index Tolerance Value
TI = Taxonomist's initials
LS = Life stage: I = Immature
P = Pupa
A = Adult

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

SLIDE INVENTORY CATALOG SHEET

Page_____ of_____

SURVEY NAME:

SURVEY CODE:

SLIDE BOX____OF____

SLOT/STATION	CS	TAXA	COMMENTS	SLOT/STATION	CS	TAXA	COMMENTS
______/______	A	________ ________ ________		______/______	A	________ ________ ________	
	B	________ ________ ________			B	________ ________ ________	
______/______	A	________ ________ ________		______/______	A	________ ________ ________	
	B	________ ________ ________			B	________ ________ ________	
______/______	A	________ ________ ________		______/______	A	________ ________ ________	
	B	________ ________ ________			B	________ ________ ________	
______/______	A	________ ________ ________		______/______	A	________ ________ ________	
	B	________ ________ ________			B	________ ________ ________	
______/______	A	________ ________ ________		______/______	A	________ ________ ________	
	B	________ ________ ________			B	________ ________ ________	

CS = cover slip

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3.5 FISH

3.5.1 DEFINITION: For the purpose of this standard operating procedure, fish shall include those vertebrate species belonging to the classes Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes), and Osteichthyes (bony fishes).

3.5.2 OBJECTIVES

1. To provide data for surface water quality standards evaluation and the National Pollutant Discharge Elimination System (NPDES) permit program;

2. to provide data to assess human health concerns with special regard to fish consumption; and

3. to provide complimentary data for assessing water quality impacts to aquatic and semi-aquatic biota.

3.5.3 FIELD SAMPLING

The collection of fish samples and field data pertaining to the objectives stated above are conducted in cooperation with the Massachusetts Division of Fisheries and Wildlife (MDFW). The MDFW supplies one full-time biologist and equipment when necessary. Fish are collected under guidelines included in a "Scientific Collecting Permit for Fish" issued to the Division of Water Pollution Control by the Division of Fisheries and Wildlife. This permit is renewed annually.

Physical Measurements

When assessing water quality impacts as stated in objective 3.5.2(3) data concerning stream reach length, width, and average depth are recorded. Substrate characteristics are visually inspected and noted. Water temperature is also recorded. Also under objective 3.5.2(3) all fish are identified, weighed, and measured. Scales or spines are sampled and used for aging. All fish are then released if they show minimal stress. Under objectives 3.5.2 (1) and (2), only targeted species of appropriate size are collected, identified, weighed, and measured. These fish are brought back to the laboratory for processing. In lakes and ponds, collection areas are marked on prepared maps, and amount of effort (time) is recorded. When electrofishing is performed conductivity is recorded along with voltage used and relative success.

Gill Netting

Gill nets are entanglement gear best described as vertical walls of netting. The typical net used by this Division is of an experimental design. The nets are 38 meters in length and two meters in depth stretched. They usually include a 1.27 cm polypropylene float line and a 23 kg lead line. The net itself is composed of five 7.6 meter monofilament panels. Mesh sizes are: 2.54 cm; 3.175 cm; 3.81 cm; 4.445 cm; and 5.08 cm.

Nets are usually set in at least 2.5 m of water and are marked by a buoy on each end. An additional buoy is attached near the center of the net in water less than 3.0 m in depth to warn boaters and/or fishermen of the obstruction. Gill nets are checked every two hours to minimize the number of unwanted fish collected. When an adequate sample size is not obtained during a typical one day set, occasionally large meshed gill nets are reset and left overnight.

Electrofishing

Electrofishing is a sampling technique in which an electric current, either alternating (a.c.) or direct (d.c.), is generated into the water to temporarily stun fish for subsequent capture. To meet sampling needs, two types of electrofishing are employed depending on the site specific situation. In areas with an adequate boat access and water deep enough for outboard motor use, an electroshock boat utilizing a gas operated generator is used. In smaller lotic situations with a bottom substrate and depth suitable for wading, a battery operated backpack electrofishing unit is applied. Using either method only those fish appropriate to the sampling scheme are netted and retained until an adequate sample size is obtained.

Trapping

Wooden cylindrical catfish traps are used to collect catfish and bullheads (Ictaluridae). These are baited, set in suitable locations, and periodically checked. The trap has an opening on one end with a cone-shaped entrance. The fish enter through the cone and cannot find the entrance once in the box end of the trap.

Field Processing

Fish are sampled using any combination of the previously mentioned techniques. Sampling is continued until sampling goals are met or until time becomes a constraining factor. All fish are kept intact and fresh in a cooler of ice and transported back to the TSB lab for further processing and preparation.

3.5.4 LABORATORY ANALYSIS

Processing

Fish collected for objectives 3.5.2 (1) and (2) are used for bioaccumulation data analysis which is incorporated into public health determinations or National Pollutant Discharge Elimination System permit reviews. Each fish is weighed whole. Length is measured from the tip of snout with mouth closed to the longest part of the caudal fin slightly compressed. This is expressed as total length.

Each fish is rinsed with deionized water and filleted. A clean, sharp fillet knife is run along each side of the backbone and then just to the outside of the rib cage. This removes a boneless fillet from each side

of the fish. The fillet is then placed, skin down, on the glass filleting surface. The knife is used to separate the flesh from the skin. Skin is discarded except when preparing trout (Salmonidae). Skin is left intact on trout because it is believed to be the most common preparation method used by fishermen. One fillet, depending on the study, is either wrapped individually, or composited with fillets from other fish of the same species and size. The opposite fillet is wrapped individually, tagged with a three or four letter code and number, and archived for future use. Samples for metals analysis are wrapped in plastic (e.g., Saran) wrap. Samples to be tested for PCB's, percent lipids, and organic scan are wrapped in household grade aluminum foil. Fillets to be analyzed for dioxin are wrapped in aluminum foil which has been rinsed with methanol and methylene chloride. The filleting surface and knife are rinsed thoroughly after each fish is filleted. Processed fish are kept frozen until they are transported to the analytical laboratory for analysis.

Fish are analyzed for metals and/or organics depending on the individual study being performed. All results are reported as mg/kg. Quality control and assurance data are recorded with each run of samples by the analytical laboratory.

Aging

All fish collected are aged by use of scales or spines. Scales are taken from various areas of a fish depending on the species being sampled. Scales are dried in scale envelopes. The impressions are made on butyrate slides, with a scale press. The impressions can then be read off a scale reader or microfilm reader. Pectoral spines are collected from Ictalurids. These spines are dried and cleaned of excess skins and flesh. They are soaked in Axion detergent, which helps loosen the skin and flesh which results in easier removal. Spines are cross-sectioned at the basal recess on a low speed diamond bladed saw. Cross-sections of .10-.20 mm. can then be read through a compound microscope. Ages are expressed as years^{+}, for example 1^{+}, 2^{+}, 3^{+}.

3.5.5 DATA MANAGEMENT

Reporting of Results

In most cases involving objectives 3.5.2 (1) and (2) results are put into tabular form and a technical memorandum is written detailing the nature of the study, methods used, and any applicable recommendations. The memorandum is distributed to interested parties including the Massachusetts Department of Public Health and the DEQE Office of Research and Standards.

Computer Files

All fish data are entered into one of 4 DBase3+ files. The files include station identification information (STAID), a record of samples (SAMPREC), the results of analyses for metals (FISHMET), and the results for organics (FISHORG). These files are linked in such a manner that data can be retrieved by species, waterbody, analyses type, concentration of contaminant, year, size, and other metrics. Data from these files are the beginning of a statewide data base.

3.5.6 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ electrofishing boat
- ☐ boat motor, gas can (and line)
- ☐ generator and gas can
- ☐ generator tote barge
- ☐ anchor, rope
- ☐ life jackets, seat pads
- ☐ fire extinguisher
- ☐ boat lights

Collecting and Sampling Gear

- ☐ backpack electrofishing gear
- ☐ pocket thermometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ plastic bags (and ties)
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ formalin
- ☐ dip-nets
- ☐ gill nets
- ☐ fish measuring board
- ☐ pan balance

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ length-weight, length-frequency forms
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ aluminum foil and plastic wrap
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice
- ☐ paper towels
- ☐ flashlights
- ☐ ear protectors

3.5.7 DATA RECORD SHEETS AND FRESHWATER AND ANADROMOUS FISHES CODING LIST

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

EXAMPLE OF SCALE (ENVELOPE)

WATERS		TOWN	
TAG NO.	SP.		NO.
TL. IN.	SL. MM.	WGT.	
SEX	M	G	
STOM.			D
Mass. F. & W. Fish Scale Record			

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

FISH COLLECTION DATA & INVENTORY

WATERBODY and PROJECT CODE:	STATION:	DATE:

WATER TEMPERATURE:	WEATHER:	METHOD of COLLECTION:	COLLECTORS:

Sample Code	Species	Length cm	Weight g	Ageing Structure	Age

Sample Type	Lab / Analysis	Samples Sent	Results Received

COMMENTS:

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

FRESHWATER AND ANADROMOUS FISHES CODING LIST

CODE	COMMON NAME[1]	LATIN NAME	ABBREVIATION
	PETROMYZONTIDAE - Lampreys		
01	American brook lamprey	(Lampetra appendix)	BL
	ACIPENSERIDAE - Sturgeons		
02	Shortnose sturgeon	(Acipenser brevirostrum)	SST
03	Atlantic sturgeon	(Acipenser oxyrhynchus)	AST
	ANGUILLIDAE - Freshwater eels		
04	American eel	(Anguilla rostrata)	E
	CLUPEIDAE - Herrings		
05	Blueback herring	(Alosa aestivalis)	H
06	Alewife	(Alosa pseudoharengus)	A
07	American shad	(Alosa sapidissima)	S
	SALMONIDAE - Trouts		
08	Coho salmon	(Oncorhynchus kisutch)	CO
09	Kokanee salmon	(Oncorhynchus nerka)	KO
10	Rainbow trout	(Oncorphynchus mykiss)	RT
11	Atlantic salmon	(Salmo salar)	AS
12	Brown trout	(Salmo trutta)	BT
13	Brook trout	(Salvelinus fontinalis)	EBT
14	Lake trout	(Salvelinus namaycush)	LT
	OSMERIDAE - Smelts		
15	Rainbow smelt	(Osmerus mordax)	RS

[1] Listed phylogenetically by family

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	UMBRIDAE - Mudminnows		
16	Eastern mudminnow	(Umbra pygmaea)	CM
	ESOCIDAE - Pikes		
17	Redfin pickerel	(Esox americanus americanus)	RP
18	Northern pike	(Esox lucius)	NP
19	Chain pickerel	(Esox niger)	CP
	CYPRINIDAE - Carps and Minnows		
20	Goldfish	(Carassius auratus)	G
21	Lake chub	(Couesius plumbeus)	LC
22	Common carp	(Cyprinus carpio)	C
23	Eastern silvery minnow	(Hybognathus regius)	SM
24	Golden shiner	(Notemigonus crysoleucas)	GS
25	Emerald shiner	(Notropis atherinoides)	ES
26	Bridled shiner	(Notropis bifrenatus)	BM
27	Common shiner	(Notropis cornutus)	CS
28	Spottail shiner	(Notropis hudsonius)	SS
29	Mimic shiner	(Notropis volucellus)	MS
30	Northern redbelly dace	(Phoxinus eos)	NRD
31	Bluntnose minnow	(Pimephales notatus)	BNM
32	Fathead minnow	(Pimephales promelas)	FM
33	Blacknose dace	(Rhinichthys atratulus)	BND
34	Longnose dace	(Rhinichthys cataractae)	LND
35	Creek chub	(Semotilus atromaculatus)	CC
36	Fallfish	(Semotilus corporalis)	F
	CATOSTOMIDAE - Suckers		
37	Longnose sucker	(Catostomus catostomus)	LS
38	White sucker	(Catostomus commersoni)	WS
39	Creek chubsucker	(Erimyzon oblongus)	CCS

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	ICTALURIDAE - Bullhead catfishes		
40	White catfish	(Ictalurus catus)	WB
41	Yellow bullhead	(Ictalurus natalis)	YB
42	Brown bullhead	(Ictalurus nebulosus)	BB
43	Channel catfish	(Ictalurus punctatus)	CB
44	Tadpole madtom	(Noturus gyrinus)	MT
	PERCOPSIDAE - Trout-perches		
45	Trout-perch	(Percopsis omiscomaycus)	TP
	GADIDAE - Codfishes		
46	Burbot	(Lota lota)	BBT
	CYPRINODONTIDAE - Killifishes		
47	Banded killifish	(Fundulus diaphanus)	K
48	Mummichog	(Fundulus heteroclitus)	M
49	Spotfin killifish	(Fundulus luciae)	SPK
50	Striped killifish	(Fundulus majalis)	SK
51	Rainwater killifish	(Lucania parva)	RK
	GASTEROSTEIDAE - Sticklebacks		
52	Fourspine stickleback	(Apeltes quadracus)	FSS
53	Threespine stickleback	(Gasterosteus aculeatus)	TSS
54	Blackspotted stickleback	(Gasterosteus wheatlandi)	BSS
55	Ninespine stickleback	(Pungitius pungitius)	NSS
	PERCICHTHYIDAE - Temperate basses		
56	White perch	(Morone americana)	WP
57	Striped bass	(Morone saxatilis)	SB

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	CENTRARCHIDAE - Sunfishes		
58	Rock bass	(Ambloplites rupestris)	RB
59	Banded sunfish	(Enneacanthus obesus)	BS
60	Redbreast sunfish	(Lepomis auritus)	Y
61	Green sunfish	(Lepomis cyanellus)	GRS
62	Pumpkinseed	(Lepomis gibbosus)	P
63	Bluegill	(Lepomis macrochirus)	B
64	Smallmouth bass	(Micropterus dolomieui)	SMB
65	Largemouth bass	(Micropterus salmoides)	LMB
66	White crappie	(Pomoxis annularis)	WC
67	Black crappie	(Pomoxis nigromaculatus)	BC
	PERCIDAE - Perches		
68	Swamp darter	(Etheostoma fusiforme)	SD
69	Tesselated darter	(Etheostoma olmstedi)	TD
70	Yellow perch	(Perca flavescens)	YP
71	Walleye	(Stizostedion vitreum vitreum)	W
	COTTIDAE - Sculpins		
72	Slimy sculpin	(Cottus cognatus)	SC

3.5.8 REFERENCES

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3.6 CAGED MINNOW TOXICITY ASSESSMENTS

3.6.1 DEFINITION: The acute impacts of both point and non-point source discharges to lotic environments can be evaluated with instream toxicity studies using caged test organisms. Upstream or control stations are necessary for comparing background levels of toxicity against levels detected at test stations. Where applicable, laboratory toxicity testing of the discharge is also recommended for the overall toxicity assessment. Although limited by length of exposure, for the purpose of this Standard Operating Procedure, caged minnow toxicity assessments are conducted with less than 14-day-old caged fathead minnows (Pimephales promelas) placed instream for a 24-hour period. A minimum of two cages/station with each cage containing 10 minnows is required. The number of surviving minnows at the end of the 24-hour study is recorded for each cage. The mean percent mortality at each station is considered the measure of instream acute toxicity.

3.6.2 OBJECTIVES

1. To characterize the magnitude and extent of acute effects of point or non-point source discharges on fathead minnows held in the receiving stream(s);

2. to provide ambient monitoring data for the 305(b) and 304(l) reports; and

3. to provide a screening tool for assessing the water quality of the Commonwealth.

3.6.3 FIELD SAMPLING

Selecting and Setting Test Stations

Instream test station locations are selected with regard to the magnitude and nature of the discharge as well as results of a mixing zone determination via an effluent dye study. In some situations, pertinent chemical sampling (e.g., amperometric titrations for Total Residual Chlorine [TRC] in μg/l downstream of a chlorinated discharge) may also be necessary for the station location process. Finally, physical characteristics such as wadable segments and velocity of the receiving stream will also dictate actual station locations.

After test stations are selected, distance from the discharge to each station is measured by either tape or rangefinder. Streamside trails may be cut where necessary to ease access to each station if a rigorous sampling schedule is planned.

Instream control and test stations are set with a pair of steel

reinforcing rods pounded into the stream bottom. The rods are aligned parallel with the stream flow approximately 0.5m apart and serve as anchors for the minnow cages. If necessary, cinder blocks can be stacked instream just below the instream test station and a plank placed from the stream edge onto the blocks. Water quality monitoring can then be conducted at each instream test station from the plank without disturbing the sediments. A dipper may be used to obtain water samples from the stream bank.

Minnow Cage Construction

Minnow cages consist of a composite of two containers; one nested inside of the other (see Figure 1). The inner container holds the minnows and is constructed with 5cm diameter acrylic plastic tube (plexiglass) with 0.5mm mesh Nytex™ screen attached to each end following the design of O'Brien and Kettle (1981). The outer container is used to reduce the water velocity flowing through the inner cage, thus protecting minnows from mechanical stress. Bottle caps may also be used to further reduce stream velocity. Outer containers are constructed from perforated, 1-liter seltzer water or soda bottles with removable bottoms. The inner cage is placed inside the outer container, after which the bottom of the latter is attached and held in place with rubber bands and paper clips.

Study Methods

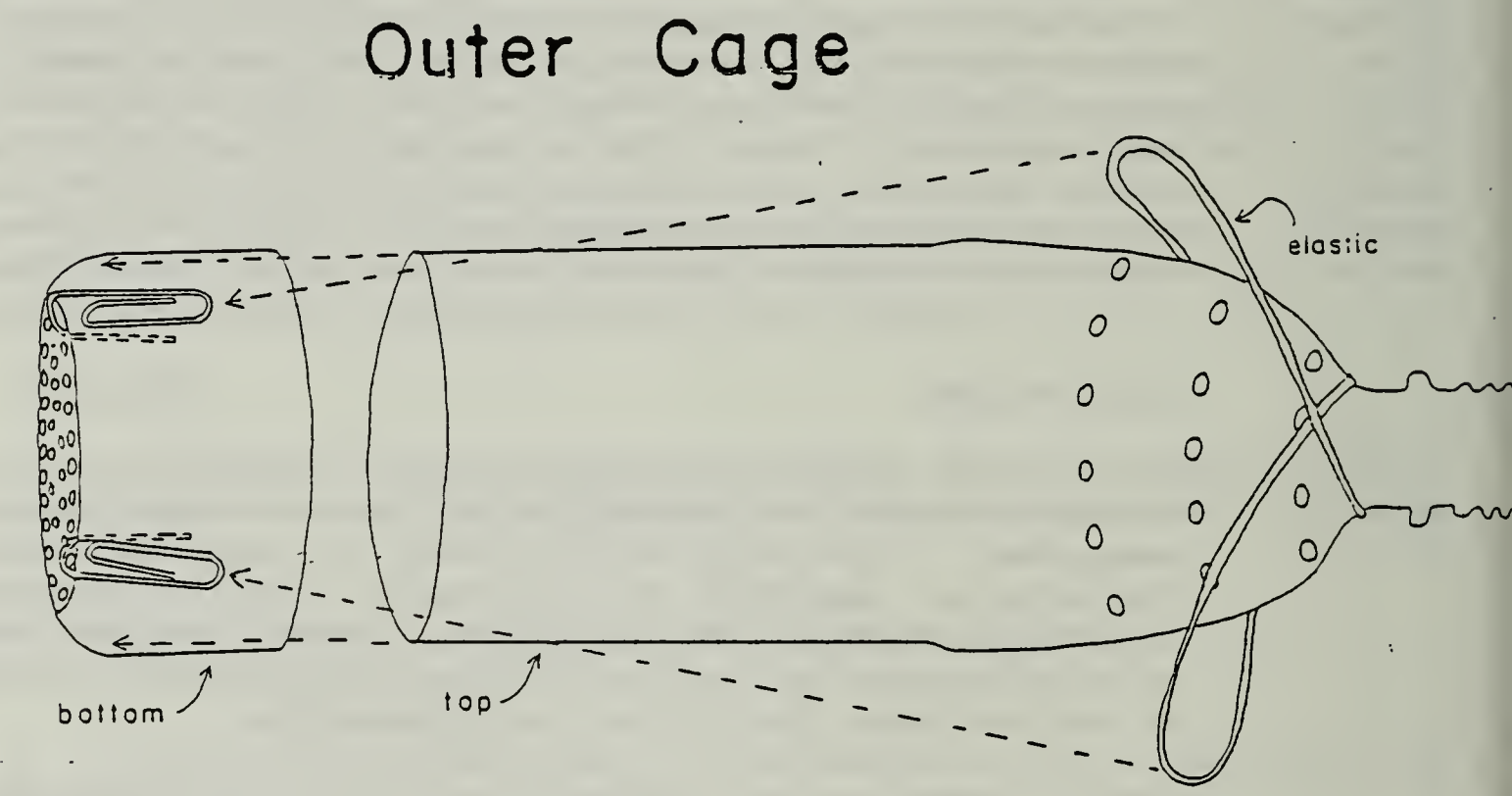
The instream waste concentration of the effluent or non-point source discharge should be determined as part of the instream toxicity assessment. Flow measurements should be taken above and below the discharge as described in the Basins Program Standard Operating Procedures (Technical Services Branch, 1989). Alternatively, instream chloride data can be used to calculate the instream waste concentration of the discharge (Szal et al., in preparation).

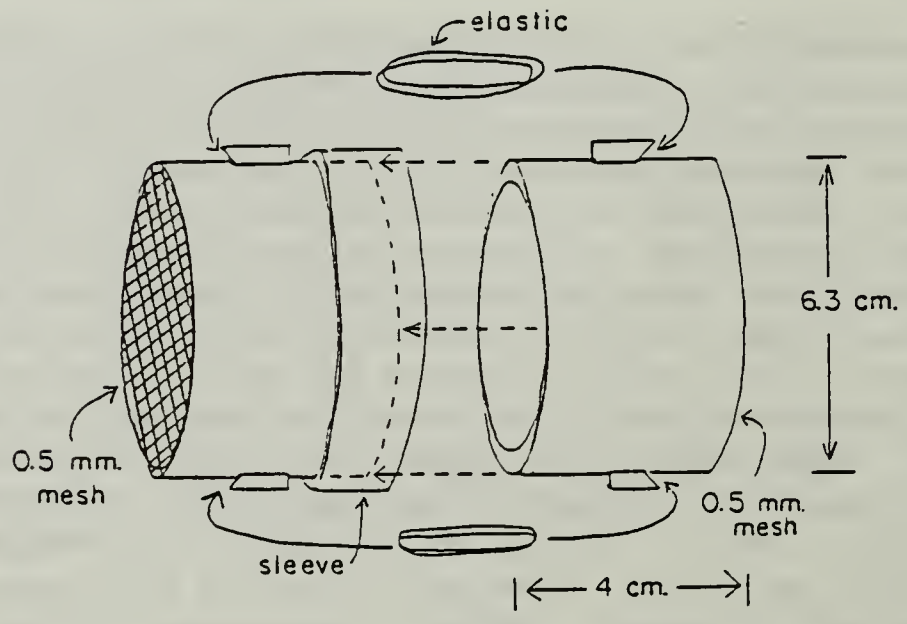
Time of travel to each instream test station is determined by dosing the effluent or a specific stream reach with rhodamine dye and recording the time at which the visible leading edge of the dye reaches each test station. This information can be used to determine site specific sampling times as well as persistence of certain wastewater constituents.

It is convenient to transport the minnows from the laboratory to the test site in a plastic bag placed in a styrofoam cooler (Jones, 1985). Minnows must be acclimated to ±1°C of the stream temperature as measured with a precalibrated hand-held thermometer. According to EPA methods, aquatic organisms should not be subjected to more than a 3°C shift in temperature over a 12-hour period (Peltier, 1978). This may be accomplished by bathing the minnow container in a cooler containing stream water until temperature equilibration is realized without exceeding the above-mentioned change in temperature. The temperature-acclimated minnows should then be randomly transferred into the inner test cages which have been set into a porcelain pan filled with approximately one inch of stream water. Minnows can either be scooped up into a small cup or caught with a wide-bore disposable pipet and distributed two or three at a time to each inner cage until all cages contain 10 minnows. Sluggish or damaged minnows should be replaced to

Figure 1. Minnow Cage Construction.
Inner and outer sections of the minnow cages used in the field experiments are depicted. The outer section, which is used to slow the velocity of water passing over the inner cage, is made from an 1-liter, plastic soft drink bottle. Inner cages with minnows are placed inside the top section of the outer cage; the latter is pushed into the bottom section and held in place with elastic bands and paper clips.

Inner cages are constructed from acrylic plastic tube (plexiglass) and Nytex^{tm} screen. The two halves of the inner cage are held together by elastic bands fitted over small plexiglass blocks which are glued to each half of the inner cage. A plexiglass sleeve is placed around the joint of the two halves to prohibit minnows from escaping should one of the elastics fail.





Inner Cage

From Szal et al., in preparation

insure quality control. Minnows should be counted twice before the top half of the inner cage is attached with rubber bands and the sleeve is positioned correctly (see Figure 1).

The porcelain pan is then submerged into a cooler containing stream water to fill the inner minnow cages with water. Air bubbles trapped against the Nytex™ screen are gently removed by tapping. Each inner cage is placed into an outer container which is subsequently secured with rubber bands and paper clips under water. Care must be taken to keep the minnow cages submersed at all times to prevent the minnows from becoming impinged upon the mesh screens.

Starting at the upstream or control station and successively working downstream, the field crews deploy a minimum of two minnow cages at each test station. The cages are wired between the rebar at a preselected distance below the stream surface (e.g., six inches). Time and date of minnow deployment is recorded on the Minnow Recording Sheets (see page 110) for each station. Several measurements are also conducted at the initiation of the toxicity test.

Temperature is taken with a precalibrated hand-held thermometer, while pH is measured electrometrically. A minimum of three velocity measurements taken with a current meter placed adjacent to the minnow cages are also recorded. Site specific sampling (e.g., TRC titrations, water quality sampling) may also be conducted at this time. Dissolved oxygen (DO) samples are also taken at each station, fixed, and titrated back at the laboratory according to a modification of the Azide Modification of the Winkler Method (Rand, 1975) as described in the Basins Program Standard Operating Procedures (Technical Services Branch, 1989). DO meters may be substituded except in the presence of chlorinated wastewater where the membrane on the DO probe may be affected by chlorine.

The instream toxicity assessments are terminated at the end of the 24-hour study period. The inner minnow cage is removed under water from the outer container. A porcelain pan is placed under the submersed inner cage and is then removed from the stream. At this time, the elastics, sleeve and the upper section of the inner cage are removed and minnows are checked for survival. Minnows not responding to gentle prodding with a pipet are considered dead. Time, date, and the number of surviving minnows is checked and recorded on the lower half of the Minnow Recording Sheets at each corresponding station. The same series of measurements taken at the initiation of the toxicity study are conducted and recorded again. Minnows should not be introduced to the stream at the end of the toxicity assessment. The screens of the inner cages can be cleaned with a toothbrush to remove sediment and minnow carcasses.

The design of the water quality sampling and analysis program for each study is determined on a site by site basis. Composite effluent samplers may be set to collect effluent during the 24 hours of study. This effluent sample can then be mixed, split and fixed as necessary for selected chemical analyses and laboratory toxicity tests. In the case of a chlorinated wastewater discharge, monitoring of TRC at instream test

stations may be warranted every 2 to 3 hours whereas an intermittent industrial wastewater discharge might only require water quality monitoring at one time during the study. Ambient toxicity monitoring with caged fathead minnows might not require any additional water quality samples other than temperature, pH, DO, and a measurement of stream velocity.

3.6.4 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard, keys
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles, ropes
- ☐ boat motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads
- ☐ brush cutter, clippers, saws
- ☐ premixed fuel
- ☐ work gloves

Field Apparel

- ☐ rain gear
- ☐ hip boots and/or waders
- ☐ disposable gloves

Collecting and Sampling Gear

Caged Minnow Studies

- ☐ pocket thermometer
- ☐ siphon tube
- ☐ <14 day old fathead minnows
- ☐ tape measure
- ☐ range finder
- ☐ ISCO™ composite samplers
- ☐ extension cords
- ☐ rebar
- ☐ sledge hammer
- ☐ flagging
- ☐ reflective tape
- ☐ large bore disposable pipets
- ☐ inner minnow cages
- ☐ outer minnow cages
- ☐ wire (coated)
- ☐ elastics
- ☐ paper clips
- ☐ white porcelain pans
- ☐ headlamps
- ☐ current meter
- ☐ large buckets
- ☐ cinder blocks
- ☐ planks
- ☐ ruler
- ☐ field recording sheets
- ☐ toothbrush
- ☐ small cup or scoop
- ☐ styrofoam cooler

Dye Studies

- ☐ stop watch
- ☐ rhodamine dye

Collecting and Sampling Gear (cont)

Water Quality Sampling

- ☐ dipper
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic bottles
- ☐ DO bottles and rack
- ☐ fixatives and preservatives
- ☐ pH buffers
- ☐ pH meter
- ☐ field notebooks, pencil
- ☐ tool kit

Chlorine Titrations

- ☐ Fisher and Porter™ amperometric titrators
- ☐ pH 4 buffer
- ☐ 5% KI solution
- ☐ PAO titrant
- ☐ Q-tips™
- ☐ Bon-Ami™ cleaner
- ☐ distilled water
- ☐ 200 ml beakers
- ☐ 1000 ml volumetric flask
- ☐ 1 ml pipets
- ☐ Chlorox™ bleach
- ☐ miscellaneous fuses

Miscellaneous Items

- ☐ coolers, ice
- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ pencils, pens
- ☐ waterproof markers
- ☐ tags and labels (with elastics or strings)
- ☐ insect repellent
- ☐ sunscreen
- ☐ camera
- ☐ film
- ☐ paper towels
- ☐ hand lens
- ☐ disposable gloves
- ☐ first aid kit
- ☐ lab goggles

3.6.5 DATA RECORD SHEET

MINNOW RECORDING SHEET

STATION

Analysts:

Parameter					
START DATE					
START TIME					
# MINNOWS DEPLOYED	|	|	|	|	|
pH					
TEMP					
VELOCITY					
FINISH DATE					
FINISH TIME					
# SURVIVORS	|	|	|	|	|
pH					
TEMP					
VELOCITY					

3.6.6 REFERENCES

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3.7 MICROTOX™ ANALYSIS

3.7.1 DEFINITION: The Microtox™ toxicity analyzer uses a lyophilized (freeze-dried) marine bioluminescent bacterium (Photobacterium phosphoreum) which, upon reconstitution, emits a fairly constant level of light. Upon exposure to a toxicant, the level of bioluminescence is diminished in direct proportion to the toxicant concentration.

3.7.2 OBJECTIVES:

1. To assess the effectiveness of the Commonwealth's municipally-owned and industrial wastewater treatment plants in eliminating or preventing aquatic toxicity;

2. to selectively screen water and sediment samples prior to performing more expensive and time-consuming conventional toxicity tests;

3. to determine the toxicity of known toxicants using laboratory-prepared solutions of known concentrations; and

4. to compare Microtox™ test results with results from other toxicity tests.

3.7.3 FIELD SAMPLING

Both grab and composite samples can be analyzed for acute toxicity with the Microtox™ Toxicity Analyzer. Generally, grab samples are taken at instream stations while composite samples of effluents are collected at municipal and industrial wastewater treatment facilities. Although the compositing technique does have an "averaging" effect (which might mask a peak of toxicity), it appears to be a good indicator of average toxicity conditions. Composite samples are collected by ISCO automatic samplers.

Sample Container Preparation

The 450 ml borosilicate-type glass containers are prepared according to the methods described in the United States Environmental Protection Agency's Handbook for Sampling and Sample Preservation of Water and Wastewaters (See: "References"), unless the containers are previously unused.

Sample Collection and Handling

The following procedure is used to collect samples for Microtox™ analysis:

1. Clean 16-ounce glass jars with teflon-lined caps should be filled completely to eliminate any headspace.

2. Samples can either be grabs or composites but must "match" samples sent for chemical analysis.

3. Both pH and Total Residual Chlorine (TRC) readings should be taken in the field and noted on the sample tag. If the sample is a composite then the TRC measurement should be taken on the composite.

4. The following parameters should be analyzed to the detection limit (mg/l) specified:

Total Hardness	0.5
Total Alkalinity	2.0
TRC	0.02
Ammonia-Nitrogen	0.1
TKN	0.03
TOC	0.5
Specific Conductance	--
Total Suspended Solids	--
Total Metals:	
Ag, Cd, Cu, Pb	0.005
Cr, Ni	0.1
Al, Fe, Zn	0.2

5. The samples should be stored on ice for transport back to the laboratory.

Sampling information noted on the sample tags include:

Source/Town:

Sampling Dates: (Both dates if 24h composite)

Sample Type: (Grab or composite)

Sampling Location: (Exact location/description)

pH:

TRC:

Upon arrival at the Microtox™ Laboratory each sample is assigned a log number and corresponding sample information is recorded in the Microtox™ notebook. Before the sample is analyzed, both pH and TRC are tested in the Microtox™ Laboratory and the results recorded. If necessary, the sample is dechlorinated with sodium thiosulfate prior to the toxicity analysis as chlorine is very toxic to the Microtox™ bacteria. The maximum holding time for a sample after collection is 24-hours.

3.7.4 LABORATORY ANALYSIS

The basic procedure for the Microtox™ system employs duplicates of both a non-toxic control and four serial dilutions of the sample. The mean response of the duplicate control is used to normalize the duplicate responses of the four test concentrations of sample when the test results are reduced. Detailed operating procedures for using the Microtox™ Analyzer are found in the Microtox System Operating Manual (see: "References").

Laboratory Equipment and Related Supplies

1. Beckman Microtox™ model 2055 toxicity analyzer
2. strip chart recorder, chart paper
3. Microtox™ reagent (lyophilized)
4. Microtox™ reagent diluent
5. Microtox™ reconstruction solution
6. Microtox™ osmotic adjusting solution
7. cuvettes, glass, disposable [11.75 mm x 50 mm in size]
8. recorder pen, black
9. Eppendorf 10 1 pipet, micropipette tips 1-100 1
10. Eppendorf 500 1 pipet, micropipette tips 101-1000 1
11. parafilm, kimwipes
12. disposable gloves
13. Hach Kit Model DR100 Colorimeter

3.7.5 QUALITY ASSURANCE

Every tenth sample is tested in duplicate to check consistency and reproducability of results.

3.7.6 FIELD EQUIPMENT AND SUPPLY LIST

Collecting and Sampling Gear

- [ ] 450 ml borosilicate type glass containers with caps
- [ ] Orion model 201 field pH meter
- [ ] rubber gloves

Miscellaneous Items

- [ ] tags, labels, elastics
- [ ] pencils, pens
- [ ] plastic wrap
- [ ] first aid kit
- [ ] cooler, ice

3.7.7 INTERPRETATION AND REPORTING OF MICROTOX RESULTS

Test Description

Microtox™ is the trade name for a particular acute toxicity test. The test is used as a toxics screening tool in addition to other, more traditional, methods of analysis.

The Microtox™ analyzer uses freeze-dried luminescent bacteria as its test organisms. When re-hydrated, these bacteria emit light. To test a water sample for toxicity using Microtox™, an analyst prepares a series of dilutions of the sample and adds re-hydrated bacteria to these. The light intensity of each sample dilution is measured at preselected time intervals over a 30-minute period and compared with that of a control (bacteria only). It is assumed that changes in light intensity are due to toxicant interference with the biochemical reaction that produces light. Toxicity is then measured as the percent decrease in light intensity of each of the sample dilutions compared with that of the control.

Data Interpretations

The most commonly used result from these tests is the 30-minute EC_{50}. This is defined as the sample Concentration causing a 50% reduction in the measured Effect (light production) over a 30-minute time period. The relationship of the EC_{50} to toxicity is an inverse one; i.e., the lower the EC_{50}, the greater the toxicity of the sample.

A useful conversion of the EC_{50} is the Toxic Unit. This is simply the inverse of the EC_{50} multiplied by a factor of 100:

$$\text{Toxic Units} = \frac{100}{EC_{50}\ (\%)}$$

Toxic Units approximate the amount of dilution a sample must undergo so as not to induce a toxic response in the test organisms (the Microtox™ bacteria). As Toxic Units increase, so does the relative toxic strength of a sample. The relationship of EC_{50}'s, Toxic Units, and toxicity are demonstrated below:

EC_{50} (%)	Toxic Units	Toxicity
0.5	200	High
1.0	100	
10.0	10	
100.0	1	Low

Samples not toxic enough to produce a full 50% decrease in light over the time allotted for the test may still be toxic enough to produce a response in the test. The EC_{20} and EC_{10} (sample concentrations causing a 20% and 10% reduction in light intensity respectively) are reported in order to give the regulator an idea of incipient toxicity - sample dilutions which induce a small, but measurable response in the test.

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

MICROTOX™ RESULTS REPORTING FORM

SAMPLES TESTED

LOG #					
SITE					
SAMPLE TYPE					
DATE COLLECTED					
DATE TESTED					
COLLECTOR					
FIELD pH					
LAB pH					
HARDNESS					
SPEC. COND.					

MICROTOX™ RESULTS

LOG #	5 MIN.	15 MIN.	30 MIN.	
				EC_{10}
				EC_{20}
				EC_{50}
				TOXIC UNITS (T.U.)

NOTE: RESULTS GIVEN AS % VOLUME OF SAMPLE

Results of the Microtox™ test are also reported for three different periods of exposure: 5-minute, 15-minute, and 30-minute. A decrease in the EC_{50} over time (increase in Toxic Units) usually indicates the presence of persistent toxicants (e.g., metals) in the sample. An increase in the EC_{50} over time (decrease in Toxic Units) suggests that non-persistent toxics (e.g., volatile, biodegradables, photo or hydrolyzible material) are present at time of sampling.

3.7.8 MICROTOX™ SEDIMENT TOXICITY TESTING

The Microtox™ bioassay can also be used to determine the toxicity of the water soluble fraction (WSF) of sediment samples. Detailed sample preparation procedures are found in the U.S. Environmental Protection Agency's draft Permit Guidance Manual on Hazardous Waste Land Treatment Demonstrations (See: "References").

Laboratory Equipment and Related Supplies

1. Eberbach shaker table - small tabletop model with carrying tray
2. IEC high speed centrifuge model HN
3. Mettler balance
4. Dessicator
5. Drying oven
6. Evaporating dishes
7. Fleaker beakers
8. Centrifuge tubes
9. Graduated cylinders
10. Tongs

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3.8 CHLOROPHYLL ANALYSIS

3.8.1 DEFINITION: Chlorophyll is a pigment found in plants that allows the organism to use radiant energy for converting carbon dioxide into organic compounds in a process called photosynthesis. Several types of chlorophylls exist and these and other pigments are used to characterize algae. One type, chlorophyll a, is measured for it is found in all algae. A knowledge of chlorophyll a concentrations provides qualitative and quantitative estimations of phytoplanktonic and periphytic biomasses for comparative assessments of geographical, spacial and temporal variations.

3.8.2 EQUIPMENT NEEDS

1. Fluorometer - either Turner 111 or the Turner Design 10-005-R field fluorometer is used. They must be equipped with blue lamp F4T5.

   Corning filter - 5-60-excitation
   Corning filter - 2-64-emission
   Photomultiplier

2. Tissue grinder and tube - Thomas Tissue Grinder

3. Side arm vacuum flask and pump

4. Millipore filter holder

5. Glass fiber filter: Reeve angel, grade 934H, 2.1 cm

6. Centrifuge (Fisher Scientific Safety Centrifuge)

7. 15 ml graduated conical end centrifuge tubes with rubber stoppers

8. 90% aqueous acetone

9. 1 N HCL

10. Saturated magnesium solution in distilled water

11. Test tube racks

12. Borosilicate cuvettes - Turner 111 - 3" cuvettes
    Turner Design - 8" cuvettes

13. Aluminum foil

14. Test tube brushes - conical end

15. Parafilm

3.8.3 LOG-IN PROCEDURE

As samples are received they are logged in and assigned a number. The samples can be frozen for further analysis, or the filter ground up for analysis the following day.

3.8.4 SAMPLE PREPARATION

Samples are generally processed as soon as they come into the laboratory, unless there are extenuating circumstances, such as faulty equipment and/or time constraints. Samples not to be analyzed within 24 hours are frozen for future analysis.

The procedure for freezing samples follows:

1) Label a 2-inch Whatman petri dish with the sample number using an indelible pen.

2) Using tweezers, take a 2.1 cm Reeve Angel, grade 934AH, glass fiber filter and place it on the Millipore filtering flask screen. Do not touch the filter. Attach the glass tube to the filter flask with the metal clamp.

3) Shake the sample well.

4) Measure out 50 mls of sample or less. If an amount other than 50 mls is used it should be recorded in the chlorophyll data book.

5) Pour the measured sample into the filter tube and turn on the vacuum. The sample should pass quickly through the glass fiber filter; therefore more of the sample should be added. If the sample is not filtering through - either because too much sediment is present or the algal concentration is too high - then less than 50 mls can be filtered. A notation is made in the chlorophyll data book which lists the amount that was filtered.

6) Unclamp the filter holder and with tweezers transfer the filter to the previously marked petri dish.

7) Cover the petri dish and wrap it in aluminum foil to keep out the light. The petri dish with the glass fiber filter is then stored in the freezer.

8) Return the sample bottle to the refrigerator if algal counts or identifications are requested.

9) Rinse the graduated cylinder and filter holder in distilled water.

3.8.5 ANALYTICAL PROCEDURE

1) Follow steps 2-6 under "Sample Preparation."

2) Filter 50 ml (or less if necessary) of sample through a glass fiber filter under vacuum.

3) Push the filter to the bottom of tissue grinding tube.

4) Add about 3 ml of 90% acetone and 0.2 ml of the $MgCO_3$ solution.

5) Grind contents for 3 minutes.

6) The contents of the grinding tube are carefully washed into a 15 ml graduated centrifuge tube.

7) Bring the sample volume to 10 ml with 90% acetone.

8) Test tubes are wrapped with aluminum foil and stored in the refrigerator for 24 hours.

9) Test tubes are taken out of the refrigerator and put into the centrifuge.

10) Test tubes are then centrifuged for 20 minutes and the supernatant decanted immediately into stoppered test tubes.

11) Tubes are allowed to come to room temperature. The temperature is recorded and the samples are poured into a cuvette (3" for Turner 111 and 8" for Turner Design).

12) The Turner 111 requires a warm-up period of at least one-half hour, while the Turner Design 10-005-R does not require a warm-up period.

13) With Turner 111, use a blank of 90% aqueous solution of acetone to zero the instrument. Open the front door of the fluorometer and put in the cuvette containing the 90% acetone and close the door. Press the start switch. The dial should move back to 0; adjustments can be made with the calibration knob. This process should be repeated as often as necessary, i.e., if the blank is not staying on zero; but no alteration should be made until a series of samples is completed.

14) The Turner Design must also be zeroed to an acetone blank. The sample holder is located at the top of the Turner Design field fluorometer and should be recovered with the black cap after the sample is put in it.

15) Readings for both the Turner 111 and the Turner Design should be within 20-80% of the scale. This can be achieved by either reducing or increasing the opening to the lamp by moving the knob on the right front of the Turner 111 fluorometer. The sensitivity levels are 1x, 3x, 10x, and 30x. The sensitivity level must be recorded in the chlorophyll data book in addition to whether the high intensity or regular door was used. After the first reading, 2 drops of 2N HCl is added to the cuvette. A piece of parafilm is used to cover the cuvette which is then inverted four times to mix the sample thoroughly. The sample is re-read and the new value recorded.

16) The procedure for the Turner Design field fluorometer is basically the same as for the Turner 111. The sample is put into the cuvette holder and the manual switch used to go from one sensitivity level to the next without opening the door. A reading of between 20-80% is still required for accuracy. Readings are taken before and after acid is added to the sample. The level of sensitivity (1x, 3x, 6x, 10x, 31.6x) must be recorded in the chlorophyll data book, as well as whether the levels were set at 1 or 100.

Calculation of Chlorophyll Concentrations

Chlorophyll concentrations are determined by using the following formulas:

$$\text{chlorophyll } (\mu g/l) = Fs \frac{rs}{rs-1} (Rb-RA)$$

$$\text{pheophytin } (\mu g/l) = Fs \frac{rs}{rs-1} (rsRa-Rb)$$

where,
Fs = conversion factor for sensitivity level "s"
rs = before and after acidification ratio of sensitivity level "s"
Rb = fluorometer reading before acidification
Ra = fluorometer reading after acidification

A computer program is used to calculate the chlorophyll concentrations for samples run on the Turner Design fluorometer. This program requires the investigator to type in the sensitivity level and the difference between the before and after acidification values is used.

During the summer of 1986 personnel of the Technical Services Branch (TSB) conducted a laboratory experiment with a Turner Design Fluorometer in order to determine the effect of pheophytin b on freshwater chlorophyll a readings. Pheophytin b is the degradation product of chlorophyll b which is the primary pigment of green algae. The Turner Design instrument measures the fluorescence of chlorophyll a as well as that of pheophytin a and b. Chlorophyll b is not read at the same frequency as chlorophyll a. The emission filter used at the TSB (Corning C/S 2-64) partially rejects pheophytin b (See: "References" - Turner Designs, 1981). It was found and recorded in various unpublished memoranda (See "References") that unless a sample had elevated counts of green algae the readings obtained prior to acidification and 90 seconds thereafter would give a reliable estimate of the concentration of chlorophyll a in an algal sample. In cases with elevated counts of green algae an annotation should be made alongside the chlorophyll a concentration stating that the concentration may reflect the presence of chlorophyll b and is probably lower than as recorded. As a result of this investigation, the TSB now presents chlorophyll data as chlorophyll a in mg/m^3.

3.8.6 INSTRUMENT CALIBRATION

Fluorometers are calibrated using chlorophyll samples provided by the United States Environmental Protection Agency. Calibrations are performed at the start of every field season and redone if any changes are made to the fluorometer such as changing the light bulb.

Samples for chlorophyll analysis are periodically split with another laboratory or run on two separate fluorometers.

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4.0 QUALITY ASSURANCE

4.0 QUALITY ASSURANCE

4.1 PURPOSE AND SCOPE

A quality assurance program has been put in place to validate both the reliability of field and laboratory techniques and the integrity of the biomonitoring data. An essential element of this program is the development of standardized field and laboratory methodologies as outlined in this manual of operating procedures. Standard methods allow for the determination of the accuracy, precision, and variability of biomonitoring data.

Although details pertaining to the quality assurance program have already been presented for individual biomonitoring program elements, major components of the program that are applicable to most biomonitoring activities are summarized in this section.

4.2 INTRALABORATORY QUALITY ASSURANCE

1) A staff of adequately trained aquatic biologists is maintained; each with knowledge of the taxonomy and pollution ecology of one or more freshwater communities. These include bacteria, algae, macrophyton, aquatic macroinvertebrates, and fish.

2) Collecting gear such as nets, sieves, and grab samplers are inspected and maintained frequently.

3) Field and laboratory equipment such as pH and dissolved oxygen meters, microscopes, and fluorometers are maintained and calibrated on a routine basis.

4) Field studies are carefully planned in advance to insure that appropriate sites are sampled and that the proper number of samples are obtained to meet survey goals and objectives.

5) All samples are clearly labeled at the time of collection, recorded in hard-bound log books, and tracked in a step-wise fashion throughout their processing in the laboratory.

6) A reference library is maintained which includes up-to-date identification manuals and keys and both benchmark and recent literature on all aspects of water pollution and its impact on aquatic life.

7) A reference specimen collection is maintained for confirming the proper identification of aquatic invertebrates. Similar collections for other communities (e.g., fish) are under development. In addition, many reference specimens and other organisms of interest are photographed and added to an extensive collection of slides to be used as taxonomic aids and for training purposes.

8) Aquatic macroinvertebrate, algae, chlorophyll, and Microtox™ data are input to computerized data storage and retrieval systems insofar as is allowed by time and personnel constraints. All data sets are carefully proofread and edited during this process. A similar system is proposed for the storage of data generated by the fish sampling program.

9) All reporting elements receive peer and/or supervisory review and numerical analyses are checked for mathematical errors.

4.3 INTERLABORATORY QUALITY ASSURANCE

1) Reference samples containing known chlorophyll *a* concentrations, predetermined phytoplankton counts, or known invertebrate taxa are routinely provided to the biomonitoring staff by the United States Environmental Protection Agency (U.S. EPA) for instrumentation calibration and evaluation of laboratory performance.

2) Occasionally biological surveys are conducted simultaneously with the USEPA or other state agencies to compare field and laboratory methods and to determine interlaboratory variability of results.

3) Specimens that present particular problems with their identification are often sent to expert taxonomists for confirmation. A separate log book is used to record the date and to whom specimens are sent, and, ultimately, the date and details pertaining to the taxonomists' responses.

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5.0 LABORATORY AND SAFETY CONCERNS

5.0 LABORATORY AND SAFETY CONCERNS

5.1 EQUIPMENT

Personnel

Masks - for organic vapors
Face Shields
Goggles
Gloves
Aprons

Lab Equipment

Fume-hood
Spill kits
Fire extinguisher
First-aid kit
Chemical waste containers
Eye Wash Stations

5.2 GENERAL LABORATORY CONCERNS

Ventilation

The TSB laboratory, which is located in the basement of the Westview Building, is equipped with a non-vented fume-hood. This fume-hood is not vented to the outside; instead, it has a filter containing activated charcoal which has a certain fixed capacity to absorb fumes from acids and solvents. To be used correctly, the front door should be down and the blower should be turned on. The fume-hood's blower and light should always be shut off after use. Chemicals should not be stored under the hood.

Handling of Chemicals

If wash bottles are used to dispense chemicals, separate the inner "pipe", located under the cap, from the upper portion before storing. This will stop chemicals from dripping out while stored. Solvents, such as acetone are extremely flammable and explosive. Care should be used when transferring solvents and other related materials from container to container.

5.3 GENERAL LABORATORY CLEAN-UP PROCEDURES

1) Keep bench tops and all work areas clear.

2) Wipe down all bench tops after use with a sponge or wetted paper towels.

3) All glassware must be washed with a phosphorus-free soap (such as Alconox™) and rinsed twice with distilled water.

4) Bottles used for algae samples should be washed with Alconox™; rinsed twice and then washed with 10% hydrochloric acid (HCI) followed by three rinses with distilled water.

5.4 HANDLING HAZARDOUS MATERIALS

Spills

Quickly rinse off in cool water any chemical to which skin is exposed. Acids should be flushed from the skin for at least 15 minutes. Spill kits for acids and alkali chemicals are located below the chemical waste storage table.

How to Use the Acid or Alkali Spill Kits

1) Contain the spill by distributing absorbent around the perimeter.

2) Sprinkle the acid or alkali neutralizer beginning at the perimeter and working inwards.

3) When the reactions begin to slow, begin mixing with the scoops provided to insure that all the acid/alkali has been neutralized.

4) Use plastic scoops to gather up the absorbent and neutralizer and transfer to plastic bags.

5) Wipe up areas with a damp sponge.

Hazardous Waste Storage

Any chemical wastes that are generated should be stored in labeled glass containers. These should be kept on the table in the laboratory which is labeled Chemical Waste Storage until full. Labels should be affixed to the bottle rather than attached with a rubber band. Transfer of waste material into a similarly labeled waste can (i.e., acids, solvents, bases) should be done if proper waste cans are available. If waste cans are not available then the labeled waste containers should be stored in the barrels provided.

5.5 LABORATORY INFORMATION BY TASK

Chlorophyll a Extraction

Avoid breathing acetone fumes as they can irritate the lining of the nose and throat. Avoid contact with skin; prolonged exposure can result in skin rashes. Different grades of acetone are used for different tasks. For chlorophyll a analysis use only those bottles marked "For chlorophyll a analysis only." Gloves and goggles, should be used when working with acetone. The area should be well ventilated; to ensure this, use the fan when necessary, or face masks which filter organic vapors.

Bottles which have held the algae/chlorophyll a samples should be acid washed before being used again. To make the 10% HCI solution, add 10 mls of concentrated hydrochloric acid to 90 ml of distilled water. Always add acid to water and not the reverse. Remember the slogan: "Add acid to water just like you oughta." Gloves, goggles and aprons should be used when working with concentrated acids.

Acetone wastes should be stored in clearly labeled bottles; do not store with nitric or sulfuric acid wastes.

Macroinvertebrate Sorting

Ethyl alcohol is used to preserve samples. Gloves and goggles should be used when handling alcohol. When samples are brought back from the field they are in the highest concentration of alcohol (95%). It is particularly important at this stage to work in a well ventilated area - usually by the sink in the lab. Alcohol is flammable; if a fire does occur in the lab use the fire extinguisher located in the hallway. Do not use water to put out the fire.

CMC Mounting medium, used for making microscope slide preparations, carries the following warning:

"[This is] a chemical in concentrated form. Avoid contact with skin or eyes. Do not take internally - may be fatal if swallowed. In case of contact with eyes flush with water for at least 15 minutes. If swallowed, and for eye contact, obtain medical attention immediately."

Fish Preservation

Fish for long-term preservation are fixed in 10% formalin before they are transferred to 50% isopropanol. Both formalin and isopropanol are irritants. Formalin, especially, can irritate the respiratory tract. Care should be taken when halding either of these chemicals. Use the fume-hood and/or face masks when making dilutions or transfering materials.

Toxicity Testing

Phenyl arsene oxide is used for chlorine titrations using the amperometric titrators. In high concentrations it is very toxic. Care must be taken to avoid inhalation of this compound. Do all titrations under the hood. Label waste containers clearly and store separately from solvents.

Unchlorinated wastewater samples are often analyzed using the Microtox™. Gloves should be worn when handling the sample containers which have often been in contact with the sample effluent, as well as when working with the sample. Gloves, pipettes, vials which have been in contact with fecal material should be promptly disposed of in a waste receptacle outside of the building.

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